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EINE DIPLOIDE FORM AUS DER TETRAPLOIDEN OENOTHERA GIGANTEA

VON *HERIBERT NILSSON*
BOTANISCHES INSTITUT, LUND

AUS *Oenothera Lamarckiana* ist in meinen Kulturen nur einmal eine tetraploide Pflanze hervorgegangen, und zwar zu Beginn meiner Versuche im Jahre 1907. Über diese Riesenrasse, die *O. gigantea* genannt wurde, habe ich mehrmals berichtet (HERIBERT NILSSON, 1909, 1912, 1915). Bei fortgesetzter Züchtung hat sich die Rasse als Riesentypus konstant gezeigt.

Im Jahre 1922 erhielt ich aber in einer Linie (76—22), die aus 57 Pflanzen bestand, zwei Pflanzen, die kaum ausgeprägt *gigantea*-ähnlich waren. Eine dieser Pflanzen (76/143) wurde von HÅKANSSON fixiert und von ihm als diploid befunden. Sie hatte auch wie *O. Lamarckiana* dreieckige Pollenkörner, nicht wie *O. gigantea* viereckige (HÅKANSSON, 1924). Die andere Pflanze (76/137) wurde geselbstet und ergab eine Nachkommenschaft (119—24), die in bezug auf die Rotnervigkeit der Blätter die Spaltung 48 : 9 aufwies, also annähernd monohybrid. Drei Geschwisterlinien vom Riesentypus, die aus 34 bzw. 41 und 38 Individuen bestanden, hatten nur rotnervige Pflanzen. Da *gigantea* wie *Lamarckiana* keine rotnervigen Homozygoten bildet, liegen hier wie gewöhnlich bei *gigantea* hohe Spaltungszahlen vor.

Die Linie 119—24 wich ebenso wie die Mutterpflanze vom *gigantea*-Typus ab. Die Rosetten schossten deutlich später als bei *O. Lamarckiana* aber früher als bei *O. gigantea* (vgl. Fig. 1 und 2). Sie hatten schmalere Blätter, fast wie *Lamarckiana*-Blätter, aber sie waren kurz wie bei *gigantea* und sehr buckelig wie bei dieser, jedoch mehr kleinbuckelig. Die Mitte der Rosette war etwas lichter grün mit einem Stich ins Grauliche wie beim *rubrisepala*-Typus. Die Blattstiele waren nicht gerade, sondern geschlängelt und gedreht, eine Eigenschaft, die weder bei *Lamarckiana* noch bei *gigantea* zu finden ist, aber für die Typen *lata* und *stricta* sehr charakteristisch ist. Der Stengel war schmal wie bei *Lamarckiana*, aber kürzer als bei dieser (Fig. 1), was bei nebeneinander stehenden Reihen sehr auffallend war. In dieser Hinsicht stimmt der Typus mit *gigantea* überein, die, obgleich in allen Organen kräftiger als *Lamarckiana*, nicht so hochwüchsig ist.

Die Knospen waren deutlich kürzer und auch etwas dicker als die von *Lamarckiana*, näherten sich in dieser Hinsicht dem *gigantea*-Typus, waren aber nicht riesengross. Auffallend war ihre Farbe, die ganz wie bei *rubrisepala*-Knospen intensiv braunrot war. Die Farbe war stärker als bei *gigantea*, die sehr stark pigmentierte Knospen hat, und sehr viel stärker als bei *Lamarckiana*. Die Blüten hatten *Lamarckiana*-Grösse, waren aber mehr gesättigt gelb, wie bei *gigantea* und *rubrisepala*.

HÅKANSSON hat an zwei Pflanzen dieses Typus Fixierungen ausgeführt. Sie zeigten in den Wurzelspitzen 14, in PMZ 7 Chromosomen,

Fig. 1. Im Hintergrund, gegen den Schirm, drei Individuen von *O. Lamarckiana*, schon aufgeblüht. Zwischen ihnen die noch nicht blühenden, kopfförmigen Gipfel von *O. diplogigantea*. (Källby, 1937).

waren also diploid. Die Pollenkörner waren wie bei anderen diploiden Oenotheren dreieckig (HÅKANSSON, 1927, 1928).

Aus der tetraploiden *O. gigantea* ist also offenbar eine diploide Form entstanden, die später einheitlich geblieben ist. Ich nenne diesen Typus *O. diplogigantea*.

Da *O. gigantea* einen Riesentypus bezeichnet, der durch Verdoppelung der Chromosomenzahl entstanden ist, so wäre am ehesten zu erwarten, dass die aus ihr rückgebildete diploide Form eine *O. Lamarckiana* sein solle. Dies ist aber nicht der Fall, wie meine Beschreibung von *O. diplogigantea* schon gezeigt hat.

Auffallend ist, dass der diplogigantea-Typus mehrere Züge von

gigantea aufweist. Kurze, stumpfe, stark buckelige Blätter, niedrigen Wuchs, spätes Schossen und spätes Blühen, kurze und ziemlich dicke Knospen, alles typische *gigantea*-Charaktere, die auf keinen anderen Mutantentypus zurückgeführt werden können. Die stark pigmentierten Knospen können auch als *gigantea*-Merkmal betrachtet werden, aber die Farbe erinnerte noch mehr an eine *rubrinervis*-Form. Auch die stärker gelbe Blütenfarbe findet man bei *gigantea* wieder, aber auch bei *rubrinervis.*

Fig. 2. Im Vordergrund Rosetten von *O. gigantea,* hinten schossende *O. diplogigantea.* (Källby, 1937).

Fasst man den *gigas*-Typus nur als ein Resultat von Chromosomenverdoppelung auf, so wäre natürlich zu erwarten, dass mit der zurückerhaltenen Diploidie auch sämtliche *gigas*-Eigenschaften verschwunden sein sollten. Dass man keine *Lamarckiana* zurückerhält, ist dagegen weniger eigentümlich. Denn es ist ja möglich, dass zwischen den beiden Chromosomensätzen ein Austausch stattgefunden hat, sodass keine strukturelle *Lamarckiana* gebildet werden kann. Man könnte dann vielleicht einen der Mutantentypen von *Lamarckiana* er-

warten. Auch dies trifft nicht zu. Die *diplogigantea* zeigt ausser *gigantea*-Eigenschaften auch *rubrinervis*- und *lata*-Charaktere. Sie ist also ein ganz besonderer Typus. Die *lata*-Eigenschaften sind auch sehr eigentümlich, da *lata* trisomisch, *diplogigantea* aber normal diploid ist.

Der *diplogigantea*-Typus ist seit seinem Entstehen ganz konstant gewesen. Nur eine Pflanze wich im Jahre 1927 durch winzigen Wuchs, schmale Blätter und nicht austreibende Knospen ab. Ein Rückschlagen zu *gigantea, Lamarckiana* oder einiger sonstigen Mutante ist niemals beobachtet worden.

Die ursprüngliche *diplogigantea*-Pflanze war rotnervig. Die später gebauten Linien haben folgende Spaltung gezeigt:

TABELLE 1. *Spaltung der Nervenfarbe bei O. diplogigantea.*

Linie	Rotnervig	Weissnervig
119—24	48	9
15—26	10	1
181—27	1	—
182—27	12	4
183—27	32	4
184—27	7	1
185—27	41	7
13—30	32	14
Summe:	183	40

Gefunden wurde also die Spaltung 183 : 40. Erwartet (nach 2 : 1) $148{,}_{67} : 74{,}_{33} \pm 7{,}_{04}$.

D/m wird also sehr hoch, nämlich $4{,}_{88}$, aber nicht höher als ich früher für *O. Lamarckiana* gefunden habe, wo die Abweichung das Achtfache des mittleren Fehlers betrug. Bei *Oenothera* ist es überhaupt sehr schwierig, ja fast zwecklos, zwischen Zahlenverhältnissen Vergleiche anzustellen, die nicht unter ganz ähnlichen Bedingungen erhalten worden sind. Denn die Elimination der *RR*-Zygoten, die eine Spaltung 2 : 1 geben sollte, wird von einer Zertation begleitet, die je nach Temperatur und Griffellänge ganz verschiedene Werte geben kann (HERIBERT NILSSON, 1915, 1920, 1923). So viel kann indessen in bezug auf die Spaltung der Nervenfarbe bei *diplogigantea* gesagt werden, dass diese innerhalb der Spaltungsamplitude von *O. Lamarckiana* liegt und sich nicht *O. gigantea* nähert.

Die Natur der erblichen Differenz des *diplogigantea*-Typus gegenüber *Lamarckiana* und *gigantea* ist nur durch Kreuzungsversuche aufzuklären. Solche wurden auch schon im Jahre 1926 ausgeführt, die gekreuzten Blüten gaben aber sehr wenig Samen und keine Nachkommen. Erst im vorigen Jahr (1937) wurden sie wieder aufgenommen, da ich *O. diplogigantea* sowohl mit einer *Lamarckiana*-Linie und meinem früher beschriebenen diffus gelbbunten *flavescens*-Typus als auch mit einer später entstandenen aberranten Form, *O. planifolia* genannt, gekreuzt habe. Letztere ist sehr *Lamarckiana*-ähnlich, hat aber längere und fast ganz ebene Blätter. Sämtliche Kreuzungen gelangen sehr gut und ergaben vollentwickelte, samenreiche Kapseln. Dieses Resultat ist auch zu erwarten, weil sämtliche Formen, wie HÅKANSSON (1927) gezeigt hat, normal diploid sind.

Diplogigantea × *Lamarckiana.* — Von dieser Kreuzung erhielt ich 1938 53 Pflanzen. Schon die Rosetten waren dadurch auffallend, dass sie einen einheitlichen Typus bildeten. Dieser kam durch die starke Buckeligkeit der Blätter *diplogigantea* am nächsten, aber die Blätter waren länger, sodass die Rosette fast so gross wurde wie bei *Lamarckiana.* Der Habitus der aufgewachsenen Pflanzen erinnerte sehr an *Lamarckiana*, die stärkere Buckeligkeit der Blätter war aber auch nun auffallend. Überhaupt kann man sagen, dass die Nachkommenschaft *Lamarckiana*-ähnlich war, aber schwachen Einschlag von *diplogigantea* zeigte. Beide Elterntypen traten also nicht auf, was ja sonst bei Mutationskreuzungen die Regel ist. Das Resultat erinnerte vielmehr an eine *gigas*-Kreuzung, wobei ein intermediärer Typus gebildet wird. Nur wurde dieser in der *diplogigantea*-Kreuzung sehr *Lamarckiana*-ähnlich. Letzterer dominierte fast ganz.

Die starke Pigmentierung der Knospen ist eine charakteristische Eigenschaft von *diplogigantea*, die in dieser Hinsicht dem *rubrisepala*-Typus nahe kommt. Eine Kreuzung *rubrisepala* × *Lamarckiana* spaltet die beiden Elternformen aus, aber ausserdem eine neue Form mit ganz blassen Knospen, die ich *pallida* genannt habe (HERIBERT NILSSON, 1915). Die Kreuzung *diplogigantea* × *Lamarckiana* gab aber nicht die beiden Eltern zurück, sondern nur einen Knospentypus, der in bezug auf Form und Farbe *Lamarckiana* am nächsten kam. Pflanzen traten auf, die wohl eine deutlich stärkere Knospenfarbe als *Lamarckiana* hatten, aber von den 6 Individuen dieser Gruppe war noch keine so kräftig pigmentiert wie *diplogigantea.* Auffallend war, dass die Färbung der Knospen sehr variabel war. Nicht nur Plus-, sondern auch Minusvarianten traten auf. *Eine ganz quantitative Spal-*

tung trat in bezug auf die Pigmentierung auf, und eine Gruppierung in *Lamarckiana* und *diplogigantea* war nicht möglich. Reine *diplogigantea*-Farbe trat wie oben erwähnt nicht auf.

Eine extreme Minusform mit fast ganz pigmentfreien Knospen bildete aber eine diskontinuierliche Komponente der Spaltung. Sie repräsentierte offenbar eine *pallida*-Form. Auch von den blassesten *Lamarckiana*-Pflanzen war sie leicht zu unterscheiden. Sie trat in einer Anzahl von 9 Individuen auf.

O. diplogigantea hat niedrigeren Wuchs als *Lamarckiana*. Unter den 6 plusgefärbten Pflanzen gab es sowohl hochwüchsige wie niedrigere, und dasselbe war in den übrigen Gruppen der Fall. Hochwüchsigkeit spaltete demnach unabhängig von Farbe und wie es schien ganz quantitativ. Dass diese Variabilität nur modifikativ sein könne, ist ganz unwahrscheinlich, denn sie ist auffallend grösser als man sie sonst bei *Lamarckiana* sieht.

Die verwendete Mutterpflanze von *diplogigantea* war weissnervig, die Pollenpflanze von *Lamarckiana* rotnervig. Die Kreuzung war also eine Zertationskreuzung, wo ein Überschuss an Rotnervigen zu erwarten war. Die Spaltung betrug 31 : 23, fiel also so aus, wie man es bei diesem Kreuzungstypus innerhalb *Lamarckiana* zu erwarten hatte.

Diplogigantea × *flavescens*. — Zwei Kreuzungen dieser Verbindung wurden ausgeführt. Beide bestätigten in den Hauptzügen die schon behandelte Kreuzung *diplogigantea* × *Lamarckiana*. *Flavescens* ist auch habituell ganz *Lamarckiana*-ähnlich, die Farbe der Blätter ausgenommen. Eine quantitative Spaltung in bezug auf Knospenfarbe, Höhe und Buckeligkeit der Blätter war also zu konstatieren. Von den 31 Pflanzen der einen Kreuzung hatte eine so intensiv rote Knospen wie *diplogigantea*, 3 hatten eine Farbe, die stärker war als bei *Lamarckiana* und 5 waren *pallida*. Von den 93 Pflanzen der anderen Kreuzung waren nur 22 so früh ausgesetzt worden, dass die Knospenfarbe beurteilt werden konnte. Im Herbst wird die Pigmentierung so viel schwächer, dass die schwachgefärbten *Lamarckiana* nicht von *pallida* mit Sicherheit unterschieden werden können. Von den 22 frühblühenden Pflanzen hatten 12 eine Pigmentierung, die um *Lamarckiana* variierte, 10 waren *pallida*. Die Mehrzahl der Pflanzen waren hochwüchsig wie *Lamarckiana*.

Diplogigantea × *planifolia*. — Von dieser Kreuzung wurden 51 Pflanzen aufgezogen. Habituell ähnelten sie fast vollkommen dem *planifolia*-Typus. Sie waren hochwüchsig, reich verzweigt, mit langen, ebenen Blättern. Sogar dieselbe Blattfarbe, die bei *planifolia* sattgrün

mit einem schwachen Stich ins Rostgelbliche sehr charakteristisch ist, zeigten die Kreuzungspflanzen. *Bis auf die Blüte dominierte also der planifolia-Typus vollständig.* In dieser Kreuzung war aber die Knospenfarbe intensiv rot wie bei *diplogigantea* und die Knospen waren kürzer und abgerundeter als bei *planifolia*, ähnelten deshalb in dieser Hinsicht *diplogigantea. In bezug auf die Knospencharaktere dominierte also diplogigantea.* Da, wie oben erwähnt, *planifolia* sehr *Lamarckiana*-ähnlich ist, ist diese Umkehrung der Dominanz gegenüber den *Lamarckiana*- und *flavescens*-Kreuzungen sehr auffallend.

Gigantea × *diplogigantea* und reziprok. — Diese Kreuzungen wurden erst dieses Jahr (1938) ausgeführt. Nur verkümmerte oder schwach entwickelte Kapseln mit einem spärlichen Samenansatz wurden erhalten. Das Resultat stimmt deshalb mit dem überein, das bei einer Kreuzung *gigantea* × *Lamarckiana* erhalten wird. Hier liegt ja auch dieselbe Differenz in bezug auf die Chromosomenzahl vor. Über diese Kreuzung kann ich also erst bei einem ausführlicheren Bericht über die folgenden Nachkommenschaften der hier behandelten Kreuzungen weiter mitteilen.

Die Entstehung von *O. diplogigantea* zeigt also, dass eine tetraploide Form kaum so konstant ist, wie man allgemein angenommen hat (Lit. bei MÜNTZING, 1936). Zwar habe ich nur zweimal ein Rückschlagen von Tetraploidie zu Diploidie unter mehreren Tausenden von *gigantea*-Nachkommen beobachtet. Liegen aber diese Varianten vor, so sind sie, wie meine Versuche zeigen, obgleich keine revertierten *Lamarckiana*, so doch bei Kreuzung mit dieser vollkommen fertil. Da sie ferner in dieser Kreuzung nicht wie die Mutanten alternative Spaltung geben, sondern eine quantitative, werden sie zur *origo* einer neuen Variabilität, die an die Stammart habituell und fertil anknüpft. *Sie tragen also in einer ungeahnten Weise zur Erweiterung der Formenmannigfaltigkeit der Ausgangsart bei.*

Sowohl das Bestehenbleiben gewisser Rieseneigenschaften bei *O. diplogigantea*, trotz der Diploidie, wie das Abweichen dieser Form von den Mutanten durch die intermediäre und quantitative Spaltung in Kreuzungen mit *O. Lamarckiana* — gerade wie *O. gigantea* — scheint dafür zu sprechen, dass die Entstehung von *O. gigantea* nicht restlos durch eine Verdoppelung der Chromosomen erklärt werden kann. Das hat ja schon DE VRIES (1912) für *O. gigas* behauptet und ich bin bei einer Analyse von *O. gigantea* zu demselben Resultat ge-

kommen (HERIBERT NILSSON, 1912, 1915). *O. diplogigantea* scheint ein guter Analysator für die Klärung dieser Frage zu sein. Sie ist auch für die Feststellung der Rolle der Tetraploiden im Prozess der Artbildung von nicht geringem Interesse, da sie zeigt, dass die Tetraploiden nicht nur selbstständige Rassen bilden können, die auch für eine ökologische Separierung geeignet sein können, wie man in mehreren Fällen gezeigt hat, sondern auch durch revertierte Diploiden eine neue Variabilität der Stammart auslösen können. Wie häufig das Entstehen dieser *Neodiploiden* ist, darüber sind wir noch ganz im Ungewissen. Ich habe durch diese Mitteilung die preliminäre Aufmerksamkeit auf das Vorkommen und die Bedeutung dieser Varianten lenken wollen.

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THE EFFECT OF COLCHICINE ON MEIOSIS IN ALLIUM

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IN an earlier paper (LEVAN, 1938) I gave an account of some experiments concerning the effect of colchicine on root mitoses in *Allium*. On that occasion I emphasized the striking selectivity of the colchicine action. Without damaging any other life processes of the chromosomes, colchicine effects a temporary inactivation of the spindle mechanism, both the exterior part working from the centrosomes and the interior centromeric part. This latter action is probably associated with the retardation observed in the division of the centromeres. The cytologically most remarkable result of the colchicine action is the origin of polyploid nuclei. The doubling of the chromosomes may be repeated several times in the same cell, if the colchicine treatment is continued. Thus during a prolongated colchicine treatment the chromosome number of certain root cells of *Allium Cepa* had increased from the normal number of 16 to more than 1000 chromosomes.

So far only two investigations have been published on the effect of colchicine on meiotic chromosomes, viz. WALKER (1938) and DERMEN (1938), who studied *Tradescantia* and *Rhoeo* respectively from this point of view. My work on the colchicine action on *Allium* has this summer also included meiosis of the pollen mother cells. The material for this study was selected from my *Allium* forms cultivated at Hilleshög. *Allium cernuum* was the species mostly used. It has somatically 14 chromosomes and normally exhibits very clear cytological conditions. It smears very nicely and gives ideal fixations with BENDA—GEITLER. In addition to the normal type of this species with 7 bivalents in meiosis (described and figured in LEVAN, 1935) another form with a ring of four was studied together with a form with 3—6 additional chromosome fragments. Other *Allium* species employed were *fistulosum*, *flavum*, *nutans* ($2n = 16$), and *odorum* ($2n = 32$).

The treatment with colchicine was made in two ways: 1. cut flower stalks were put in colchicine solutions, and 2. whole inflorescences were immersed in the solutions, after the spathal leaves had been removed. In the former case only the first stages after the colchicine treatment

could be studied, in the latter case, on the other hand, the colchicine effect could be followed right through meiosis until the division of the microspore.

In the first series of experiments the following concentrations were tried: 0,01, 0,1 and 1,0 % for periods of 24, 48, and 72 hours. Different species of *Allium* were found to have a somewhat different sensitivity to colchicine: No disturbances at all occurred in 0,01 and 0,1 % even after a period of treatment lasting 72 hours. In a 1,0 % solution disturbances were found in *Allium nutans* and *fistulosum* after a period of 24 hours, while *Allium cernuum* required 48 hours in a 1,0 % solution in order to be evidently disturbed. Whether there is really a difference in the reaction to colchicine between the species cannot be decided by these experiments. The observed differences may just as well be due to a different rate of the absorption and the translocation of the colchicine within the flower stalks.

In the second series of experiments, where growing inflorescences were immersed in the colchicine solutions, only one concentration was used, viz. 1,0 %. The period of treatment was 2, 4 and 8 days. Most descriptions and figures in the present paper refer to *Allium cernuum*, after a treatment of 4 days. This treatment gave quite evident disturbances without producing any immediate lethal effects. At least some of the flowers in each inflorescence survived this treatment and formed ripe pollen. After the longer period of treatment, 8 days, several of the inflorescences were killed at once, the part of the stalks just below the flowers becoming shrunk. Putrefaction was often met with in the flowers treated with the colchicine solution. All the treated material of *Allium flavum* was lost in that way.

The most important factor as far as the consequences of the colchicine treatment are concerned is the stage of meiosis going on at the time of treatment. In the following special chapters a detailed account is given of the cytological effect of colchicine on 1. stages earlier than metakinesis, 2. the first and second divisions, and 3. postmeiotic stages.

At first, however, a short survey of a concrete experiment may be given. A large tuft of *Allium cernuum* was moved from the field into the laboratory on 19th June and about 20 of its inflorescences were treated with a 1 % solution of colchicine. After 4 days the treatment was interrupted, and the inflorescences were rinsed in destilled water. They then contained all stages of the microsporogenesis from very young archesporial cells to uninucleate pollen grains. Aceto-carmine preparations were examined daily until the 12th day after the end of

the treatment. Further, material was fixed on the 1st, 3rd, 5th, 7th and 12th day and stained in gentian violet.

From the first to the third day chief attention was directed to the inactivation process of the spindle of the first and the second division. On the 5th day, when the spindle began refunctioning, the first division showed several abnormalities of another type: an increased frequency of univalents, abnormal forms of bivalents and so on. Whole pollen chambers now contained uninucleate pollen grains exclusively of dyad or monad type, as a result of the inhibition of the meiotic cell divisions. Of course, there were also found all kinds of abnormal transitions between monad, dyad and tetrad pollen. On the 7th day the first division showed a more normal course, and the dyad pollen was entering the first microspore division. On the 9th day the pollen monads, too, were found to be dividing. At the same time tetraploid and single octoploid pollen mother cells were seen at early prophase stages, and on the 12th day these cells had reached metaphase—anaphase I. Then all pollen monads had finished their division and in younger stages of the pollen tetrads again predominated, often with sensationally abnormal forms.

I. THE EFFECT OF COLCHICINE ON PREMEIOTIC DIVISIONS AND ON MEIOSIS UP TO METAKINESIS.

The divisions of the archesporium are affected by the colchicine in the same way as other somatic divisions, *i. e.* chromosome doublings are induced. If only one division is influenced tetraploid pollen mother cells originate, if two divisions are affected octoploid cells are formed. On the 9th day after the treatment pachytene cells of three different sizes were seen, normal diploid cells, tetraploid and octoploid cells. Later on the other meiotic stages could be studied in the giant cells. Multivalent formation was observed at metaphase I, and at anaphase I 7, 14 and 28 chromosomes could be counted in the different types of cells, sometimes in the same pollen chamber. In the doubled cells at metaphase I a certain after-effect of the colchicine was frequently noted. The bipolarity of the spindle was thus changed to a multipolarity, in a similar way as that described earlier in somatic cells. In agreement with this the subsequent interkinesis of the largest cells was sometimes 3- or 4-nucleate.

The action of colchicine on the earlier stages of meiosis is difficult to interpret exactly. At pachytene unpaired threads were often seen

just after the end of the treatment. This condition agrees well with the very low chiasma frequency regularly observed on the 5th—7th day in metaphase I of *Allium cernuum*. Then entire pollen chambers are found containing very few paired chromosomes. An instance of

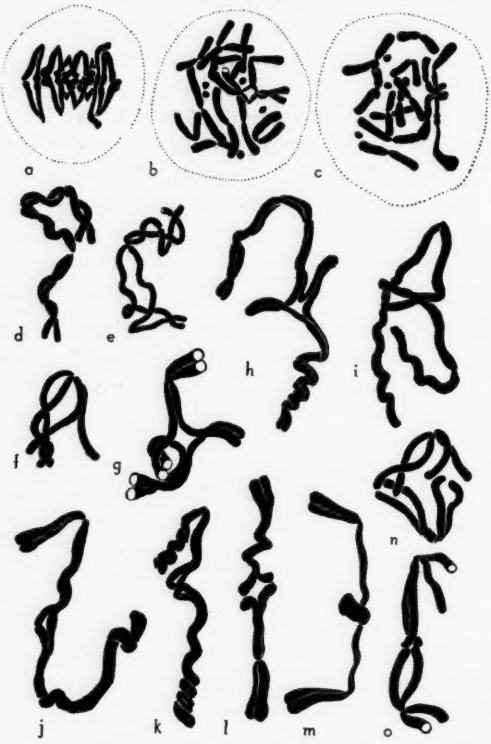

Fig. 1. *a*: untreated metaphase I, *b*, *c*: c-treated metaphase I, 5 days after the end of the treatment, high frequency of univalents, *d—f*: abnormal univalents, *g—m*: abnormal bivalents at metaphase I, *n*, *o*: ditto at anaphase I. — *a—c*: × 1100, *d—o*: × 2200.

this is furnished by the following frequency of bivalents counted in one slide:

Number of bivalents:	0	1	2	3	4	5	6	7	Total
» » cells:	8	7	5	4	5	2	0	0	31

Thus in 8 cells out of 31 all the chromosomes were present as univalents, and in no cell were as many as 6 bivalents found. This situation is in

marked contrast to the control plants, which very seldom show any univalents. In the untreated plants the number of chiasmata is high and the absolutely commonest bivalent form found is the ring-shaped bivalent with at least two chiasmata. Fig. 1 *a* shows a normal cell with its 7 bivalents, while Fig. 1 *b* and *c* are instances of these abnormal metaphases.

The few bivalents occurring in treated material are usually rod bivalents with only one chiasma, but also rings with as many as 4 chiasmata have been seen. The univalents resemble somatic chromosomes and their insertion constrictions are plainly visible. At this stage the two chromatids of each univalent are lying close together and only seldom is there any fissure seen between them.

The univalents are scattered out at random all over the cell, and when the spindle starts functioning, they do not congregate in the equatorial plane. The bivalents, on the other hand, are arranged at the equator with the centromeres extending towards the poles. At the same time the univalents begin to move into two groups orientated nearer the poles. In most cases the univalents show a clearly bipolar arrangement already before the bivalents have separated. Although irregular anaphases occur, most of them are strictly bipolar at this stage. Chromosome numbers 7—7, 6—8, 5—9 are counted at the poles and show a rather commonly occurring irregular distribution of the univalents. Since the univalents proceed directly to the nearest pole without first going to the equator their original position at the onset of the spindle action seems to be decisive for their further destiny.

After having gathered at the poles, all the chromosomes assume an anaphase appearance. The chromatids of the univalents also become widely apart. Even at this stage a delayed relational spiral is sometimes seen to uncoil (Fig. 1 *f*).

The abnormal course of the first metaphase just described goes on only for a short time. Already on the sixth day the number of free univalents has markedly decreased. On the 7th day the 7 ring bivalents are again recognised in most cells. The abnormally increased frequency of univalents is thus caused by the colchicine during a transient period of meiosis.

Another irregularity observed at metaphase I a few days after the treatment is an abnormal decrease in contraction of the bivalents. Fig. 1 *g—m* constitute examples of such bivalents. Most frequently all the cells of a pollen chamber are characterised by this peculiarity. It often occurs together with the decreased chiasma frequency. Most ex-

treme in type are the abnormal rod-shaped bivalents, which have only one chiasma. The free arms are then widely extended and clearly show the relational coiling together with the major spiral. These bivalents are contracted somewhat during metaphase—anaphase but not at all in a normal degree. Fig. 1 *n—o* shows anaphase I of two such bivalents.

This decrease in metaphase contraction was very striking and unmistakable, especially when compared with the condition of the control plants. And it must be considered to be caused by the colchicine treatment. Single cells with abnormally long and thin meiotic chromosomes are reported from *Fritillaria* (DARLINGTON, 1936, p. 308, Fig. 11) and *Tulipa* (UPCOTT, 1937, p. 359, Fig. 29). The abnormal *Allium* bivalents present a striking resemblance to these cases. In *Allium* the abnormal bivalent shape must be due to the change in the external conditions induced by the colchicine.

The finding of the abnormalities described above, viz. the increased frequency of univalents and the decreased metaphase contraction, shows that the colchicine can produce cytological effects of quite a different type from those hitherto observed. It is by no means certain, however, if colchicine acts with the same selectivity in these cases as in the inactivation of the spindle function. The prophase-induced abnormalities are perhaps symptoms of a more general poisoning of the cells.

II. THE EFFECT OF COLCHICINE ON THE FIRST AND SECOND DIVISIONS.

If metaphase or anaphase stages of the meiotic divisions are affected by the colchicine treatment, the effect will be especially striking. A description of the effect on the first division will be given first.

The terminalisation of the chiasmata in the diakinesis- and metakinesis-bivalents proceeds normally. The chromosomes are contracted and the central loop of the ring bivalents grows larger. The transition to metaphase I brings about a still greater contraction of the bivalents, accompanied by the disappearance of the nuclear membrane. Now it becomes evident that the spindle apparatus does not function, because the bivalents remain scattered around in the plasm without being arranged into an equatorial plate.

Then follows the separation of the bivalents, which in this case is of fundamental interest from a chromosome mechanical point of view,

since it offers a long-sought-for possibility to determine how much of the normal appearance and behaviour of the bivalents are due to the spindle action and how much is directed autonomically by the bivalents themselves. As seen from Fig. 2 *a—n*, the bivalents never take on a normal metaphase appearance after colchicine treatment (compare

Fig. 2. The development of the c-bivalents, *a—g*, *h—n*, *o—u*: the bivalents of three cells, representing three successive stages of metaphase I, *v—dd*: the centromeres have divided, *ee—hh*: the transition to the resting stage. — × 2200

the normal appearance Fig. 1 *a*). The tension and repulsion between the two centromeres of each bivalent, which normally cause the extension of those chromosome parts, situated between the centromeres and the chiasmata, occasionally into rather thin threads, is completely absent. Instead the bivalents retain a diakinesis-like appearance, and when the component chromosomes finally separate, this comes about exclusively by the body-repulsion. Before then the chromatids of the

different chromosomes become very clearly visible and independent and they gradually lose their shape, forced upon them by the association into bivalents. When the two daughter chromosomes separate, they are therefore cruciform without angles or turns (Fig. 2 *o*—*u*). This is in striking contrast to the appearance of anaphase chromosomes of untreated material, where the earlier arrangement of the chiasmata may be seen from the shape of the anaphase chromatids. This difference between treated and untreated material is evidently due to a delay in the separation of the bivalents, caused by the inactivation of the spindle function.

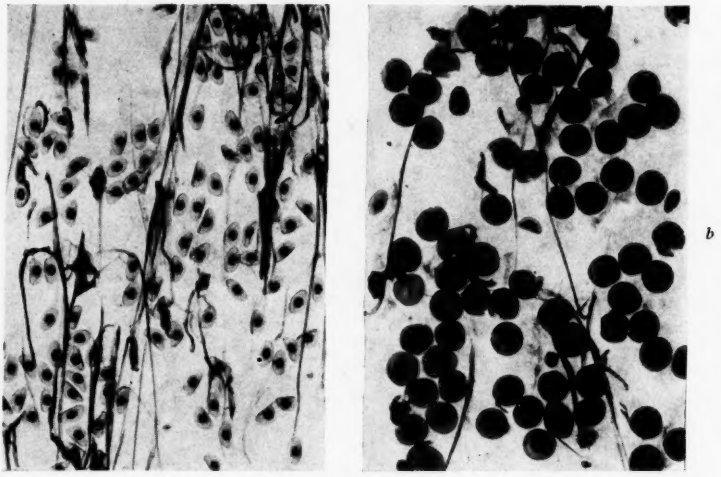

Fig. 3. *a*: normal tetrad pollen, *b*: monad pollen. — × 150. — Microphoto Otto Mattsson.

After separating, the two chromosomes of each pair remain in the vicinity of each other, and their further development exhibits some peculiar features. Instead of going into telophase—interkinesis the anaphase chromosomes retain their anaphase appearance and their centromeres are immediately divided. The half-chromosomes remain close together and their centromeres are located opposite to each other (Fig. 2 *v*—*y*). Each bivalent thus gives rise to 4 chromosomes. Their relative positions indicate for a long time which 4 chromosomes originate from the same bivalent.

After the division of the centromeres has taken place the chromosomes begin to lengthen and the spirals uncoil (Fig. 2 *z*—*hh*). The

stainability of the chromosomes is rapidly lost and the chromosomes often appear divided in ordinary fixations. In other fixations, however, where the spiralisation is clearly seen, the spirals always appear single. I therefore cannot assume that the apparent doubleness of the telophase chromosomes is real.

The spirals gradually uncoil and the nuclei enter a resting stage of long duration. This resting stage is exceedingly characteristic of colchicine treated material. Thus the tetrads of untreated material here correspond to monads. This process, the formation of monads, takes

Fig. 4. Metaphase of the first pollen division in *a*: tetrad, *b*: dyad, *c*: monad pollen. — × 2200.

place without exception in all the pollen mother cells of those pollen chambers affected by the colchicine at the sensitive period. Although both WALKER and DERMEN (l. c.) have observed the formation of monad pollen, this process evidently takes place much more regularly in *Allium* than in their material. WALKER makes the observation that in her material the first division may be eliminated by the colchicine, whereas in the same cells the second division may take place regularly. This has not been noted in *Allium*. In *Allium*, if the first division is affected by colchicine, the centromeres of the anaphase chromosomes always divide immediately in the manner just described, without first

entering interkinesis. The probable significance of this condition from a chromosome mechanical point of view will be discussed in the last chapter.

On or after the third day following the treatment a number of pollen chambers are always found to contain exclusively monad pollen. These monads are different in shape from the normal crescent or ellipsoid pollen grains. The monads are spherical and have a larger diameter than the longest axis of the normal pollen (Fig. 3). On the

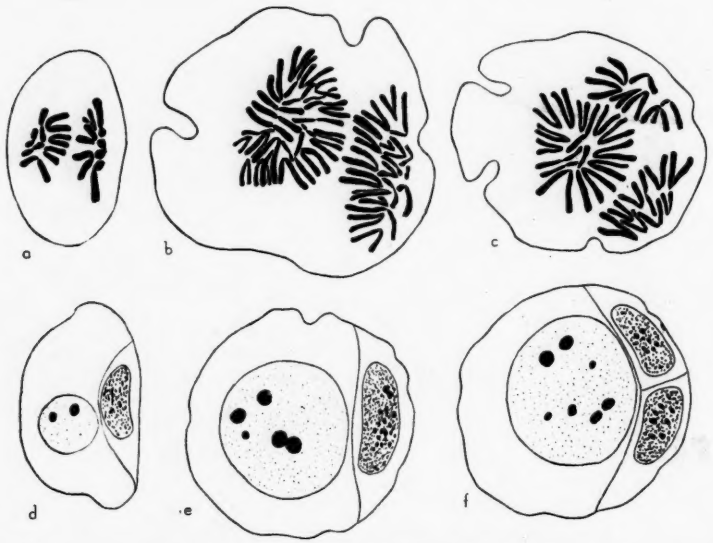

Fig. 5. *a—c*: anaphase, *d—f*: telophase of (*a*, *d*) tetrad pollen, (*b*, *e*) monad pollen, (*c*, *f*) monad pollen with tripolar spindle. — × 1100.

seventh or eighth day the monads begin to prepare for the first pollen division, their chromosomes contract and get more definite outlines. The mitoses take place on the 9th day. These mitoses are not always quite regular, many of them show an after-effect of the colchicine treatment. There are, however, many normal bipolar mitoses, and in these the appearance and number of the chromosomes may easily be studied. As expected, the number is $4x = 28$. In Fig. 4 *c* there are also six fragments present, corresponding to three fragments in somatic cells. The arrangement of the chromosomes in pairs is especially evident in the case of the fragments (Fig. 4 *c*). Each pair of chromosomes in this

metaphase corresponds to the two chromatids of one first anaphase chromosome.

Fig. 5 shows the marked asymmetry of the anaphase spindle both in normal tetrad pollen (*a*) and in monad pollen (*b*). The chromosome group, which gives rise to the vegetative nucleus, is more rounded and remains in the middle of the cell, while the other group, which gives rise to the generative nucleus, is flattened and lies close to the wall. On the tenth to eleventh day the result of the division becomes visible: one large vegetative nucleus lying in the middle of the cell, while a small lentiform quantity of plasm containing the still darkly stained generative nucleus is formed in the vicinity of one wall (Fig. 5 *d*, *e*). A comparison between the monad and tetrad pollen in the binucleate stage shows the vegetative nucleus to be considerably larger in proportion to the generative nucleus than is the case in the tetrads.

A technical detail, which made the study of the monad pollen rather difficult, is worth mentioning. While tetrad pollen as well as dyad pollen have at least one plane surface, which in smearing sticks to the slide, the monad pollen is completely spherical. This causes great losses of cells in the preparation of slides.

Besides the regular divisions of the monad pollen there were, as mentioned above, other numerous divisions affected by the colchicine. This after-effect of the colchicine was, it is true, rather weak and no c-pairs or restitution nuclei were seen. But multipolar divisions were frequently observed. Of a certain interest is that this type of anomaly in certain pollen chambers took a rather regular character. Thus in whole pollen chambers all the cells exhibited a regular tripolarity. The asymmetry of the spindle is maintained also in these tripolar divisions. At the anaphase the chromosomes were always distributed into one large centrally situated group and two small ones situated close to the wall (Fig. 5 *c*). This hardly ever results in the formation of two vegetative and one generative nucleus, two generative nuclei being always formed instead. The relative position of these two nuclei offers a further indication of the stability of this anomaly: they are situated close together and their shape is such that they resemble one normal generative nucleus cut into two (Fig. 5 *f*). In very rare cases the two generative nuclei were formed opposite each other with the vegetative nucleus situated between them.

From these anomalies certain conclusions may be drawn concerning the immediate cause of the asymmetry of the spindle in the pollen division. The nuclear type is evidently determined by the location in

the cell, the nucleus or nuclei lying close to the wall being lentiform and becoming generative nuclei. The largest group of chromosomes remains in the centre of the cell for spatial reasons, and other small groups of chromosomes are pushed against the wall and form generative nuclei. It is evident that this mechanism with two or more generative nuclei involves a reduction of the chromosome number comparable to the conditions found in root mitoses during the recovery of the spindle after colchicine treatment (LEVAN, 1938, Fig. 5).

The effect of colchicine on the second meiotic division, after the first division has taken place regularly, is often difficult to follow. The sensitive stage is probably of shorter duration and the fixations of the second division are often inferior, partly due to the crowding of the chromosomes within the dyad cells. I have, however, obtained two good slides of this stage and have been able to follow the effect of colchicine right through the second division.

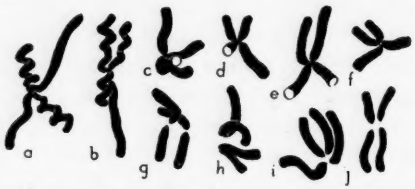

Fig. 6. The c-pairs of the second meiotic division. — × 2200.

The effect of the colchicine on this division is of the same type as that in somatic cells, *i. e.* the two chromosome halves are joined for a long time as c-pairs (Fig. 6 *a*—*f*), and when the centromeres are finally divided the half-chromosomes remain in the neighbourhood of one another (Fig. 6 *g*—*j*) and proceed in this position into the resting stage.

One marked difference in the chromosome cycle is, however, found between the second division and a common somatic division. The half-chromosomes of the second division are entirely separate right from the first anaphase and touch each other only at the centromeres. They are not involved in any relational spiral. The spiralisation often visible during this stage (Fig. 6 *a*, *b*) is a relic major spiral individual of each half-chromosome. The loop stages, characteristic of the c-pairs of somatic mitoses, during which the relational spiral is cancelled out, are thus missing at the second division. The c-pairs of the second division become cross-shape from the beginning.

The result of the c-mitosis of the second division is the formation of dyads. Fig. 4 *b* shows a metaphase of one such dyad pollen grain. The chromosome number is $2x = 14 + 4$ fragments. The colchicine treatment of the second division has cytologically the same effect as the

gene-controlled monokinetic meiosis found in several *Allium* species (LEVAN, 1936).

III. THE EFFECT OF COLCHICINE ON THE FIRST POLLEN DIVISION.

The first pollen division is also converted into a typical c-mitosis under the influence of colchicine. This could be observed in the slides until the 6th day after the termination of the treatment. After the 6th day a few colchicine-induced abnormalities remained, it is true, but the pollen division was, on the whole, normal. Owing to the presence of only the haploid number of chromosomes, the pollen grains show a very clear and schematic picture of the c-mitosis. In Fig. 7 are collected typical instances of c-pairs from four different stages of the c-mitosis;

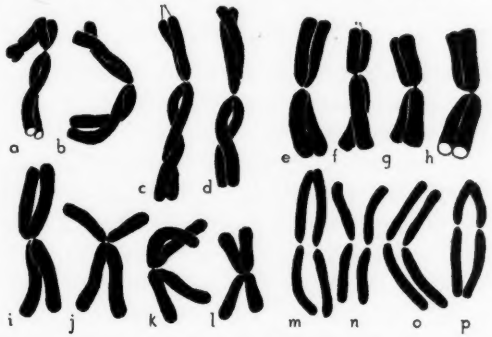

Fig. 7. The development of the c-pairs of the first pollen division, *a—d, e—h, i—l, m—p* represent four successive stages. — × 2200.

a—d represent an early stage, where the relational spiral is still very conspicuous. In *e—h* the contraction of the chromosomes has reached its maximum and the relational spiral has almost entirely uncoiled. Fig. 7 *i—l* shows the next stage, where the half-chromosomes repel each other and the c-pairs become cruciform. This stage, which is invariably found in treated root mitoses of *Allium Cepa* and *fistulosum*, is rather scarce in the pollen division of *Allium cernuum*. It is caused by a retardation of the division of the centromeres, and since it is rarely found in these pollen grains, the conclusion may be drawn that the retardation of the division of the centromere after colchicine treatment is less pronounced in the case of the pollen than in the root tips. As I have not made a parallel investigation of pollen and root tips of the

same *Allium* species it is impossible to decide whether differences in the reaction against colchicine exist between the species, or if the difference is caused by some dissimilarity in the chromosome cycle between the pollen grains and the root cells, as, for instance, the greater degree of contraction of the pollen chromosomes. It is important, however, to note that the scheme of the course of the c-mitoses, found during the study of the root mitoses is not generally valid, but may be modified in certain cases. In the above case, for instance, a very characteristic stage of the c-mitoses is usually missing.

Finally, the c-pairs after the division of the centromeres are drawn in Fig. 7 *m—p*. After this stage all the chromosomes are included in one nucleus. Thus diploid, uninucleate pollen grains are formed of the same exterior as the haploid tetrad pollen grains.

IV. DISCUSSION.

A comparison between the action of the colchicine on mitosis and meiosis reveals the fact that the main effect is the same, viz. an inactivation of the spindle apparatus. Differences occur, however, and they are caused to a great extent by the characteristic properties of the sporogenesis. These differences may be classified into two groups: they are due partly to the constitution of the reproductive organs, and partly to the behaviour of the chromosomes at meiosis.

In somatic tissues the mitoses are scattered out at random and the different stages of mitoses are intermingled irregularly. The action of colchicine on such a tissue brings about scattered cells with doubled chromosome number, and the tissue develops into a diploid—polyploid mosaic.

In the pollen mother cells the situation is entirely different. The different stages in the sporogenic tissue are more uniformly controlled by the surrounding cell environment than ordinary somatic mitoses, and the development of the cells is usually rather synchronized. And if the whole pollen chamber is not at exactly the same stage of meiosis a continuous series of different stages follow each other in the longitudinal direction of the pollen chamber.

This condition brings about a greater uniformity in the effect of the colchicine right through the sporogenic tissue. The colchicine treatment results in the whole pollen chamber being either unaffected or changed in the same way. Transitional cases occur of course, when normal and affected cells are mixed in the same pollen chamber. They

show that the sporogenic tissue at the time of treatment was in a transitional stage between a sensitive and an immune phase.

This uniformity in action and the absence of morbid changes give the colchicine effect a resemblance to the action of genes. If the result of a meiosis, treated with colchicine, is considered alone, it is in fact often impossible to decide whether it is the question of treated material or material containing genes for asynapsis, monokinetic meiosis or some other gene-controlled meiotic abnormality. This touches upon one of the most interesting sides of the biological action of colchicine. The difference between the effect of colchicine and that of chloral hydrate and other toxic substances, often employed for producing disturbances in the chromosome function, is evident. Such poisons are not specialized but generally have a depressing effect on the viability of the cells. The chromosome doublings, which are certainly sometimes brought about by them, are effected more by chance as a consequence of the general toxic effect.

Other effects induced on meiosis by the colchicine are conditioned by the special life cycle of the chromosomes during meiosis, for instance, the pairing of the chromosomes and the formation of chiasmata. The striking increase in the number of the univalents observed a few days after the treatment must be due to a checking of the chiasma formation caused directly or indirectly by the colchicine. There is present an induced asynapsis of a similar kind to those cases of asynapsis which have occasionally been observed after other environmental influences, for instance, extreme temperatures. The appearance of the univalents suggests that chiasmata have never been formed rather than that chiasmata already present have disappeared precociously. DERMEN also pictures one pollen mother cell of *Rhoeo* with exclusively univalents (l. c., Fig. 5 A), fixed 4 days after the treatment. He adds: »Beginning about the fourth day after the treatment, meiotic chromosomes appear in shorter, broken chains or in univalent form» (p. 218). And WALKER found in *Tradescantia* microspore mother cells with 12 univalents (l. c., Plate 1, Fig. 6, four days after treatment). Evidently both in *Rhoeo* and *Tradescantia* a given period of meiosis responds to the treatment with a decrease in the chiasma formation.

Colchicine treatment of the first metaphase in *Allium* brings about the peculiar phenomenon described above, that the two meiotic divisions are combined into one process, and they are not separated by any stage of despiralisation. Fig. 8 gives a schematic representation of this abnormal meiosis as compared with normal meiosis, normal mitosis and

c-mitosis. Although little is known of the conditions at the division of the centromeres, DARLINGTON must be considered correct in his assumption (1937 et alii) that a polarisation of the centromeres must precede their division. This polarisation determines, according to DARLINGTON, the orientation of the metaphase chromosomes at mitosis and the absence of polarisation causes the special type of orientation characteristic of bivalents (the »co-orientation»). Thus the difference between normal and c-meiosis may be expressed as a difference in polarisation of the centromeres: the centromeres of the c-bivalents are

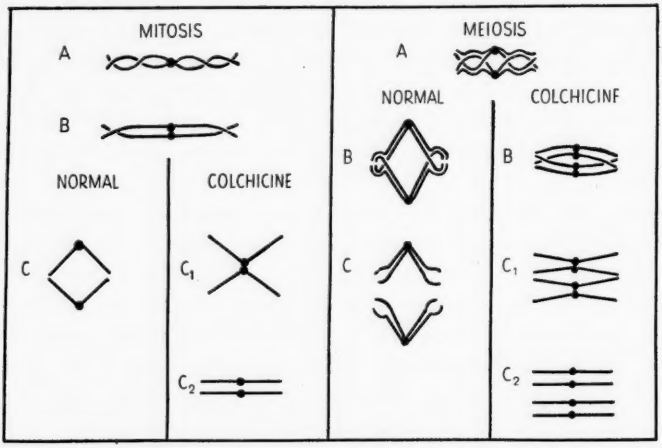

Fig. 8. Scheme of the development of the chromosomes at mitosis and meiosis in normal and c-treated *Allium*.

polarized and each daughter chromosome is therefore comparable *not* to a normal anaphase I chromosome but to a somatic c-pair (Fig. 8 C_1).

The comparison between normal and c-bivalents shows that the pressure of the interior centromeric spindle, which at mitosis does not act until after the division of the centromeres, in normal bivalents acts much earlier, viz. as soon as the bivalents begin to arrange themselves in the equatorial plane. Possibly the absence of this pressure within the c-bivalents is responsible for their centromeres becoming polarized. This pressure is absent in normal mitosis, in c-mitosis, in c-meiosis, and in the secondary equatorial plate consisting of univalents normally occurring in triploid meiosis. In all these cases division of the centromere follows.

Finally, the following survey may be given to show the con-

sequences of treatment of different stages of the microsporogenesis of *Allium cernuum*:

	Treated stage	Result
Archesporium	Division 1 *and* 2	Octoploid P.M.C.
	Division 1 *or* 2	Tetraploid P.M.C.
P.M.C.	Resting stage	No effect
	Prophase stages	Asynapsis, abnormal bivalent shape
	Division I and II	Tetraploid pollen monads
	Division II	Diploid pollen dyads
Pollen	Resting stage	No effect
	First division	Diploid nuclei in tetrad pollen

SUMMARY.

The present work is a study of the chromosome behaviour during meiosis of *Allium* after treatment with colchicine. In addition to the special effect of colchicine on the spindle mechanism, an effect on chiasma-formation and on the spiralisation was observed. The divisions of the archesporium, the second meiotic division, and the pollen division were converted into c-mitoses under the influence of colchicine.

If the first meiotic division is influenced by the treatment, meiosis takes a peculiar course called c-meiosis: the bivalents separate and the centromeres of the anaphase chromosomes divide immediately. Thus instead of pollen tetrads, monads with the tetraploid chromosome number are formed. The result of the regularity of this process is that whole pollen chambers will contain exclusively monads, and gives the impression of a gene action rather than a toxic effect.

It is suggested that the supposed polarisation of the centromeres of the c-bivalents is due to the absence of the interior spindle, which is active in untreated bivalents.

Hilleshög, September 1938.

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THE CHROMOSOME NUMBERS OF THREE ARTEMISIA FORMS

BY *STELLAN ERLANDSSON*
STOCKHOLM

A form of *Artemisia,* occurring on sandy and stony ground on the coast at the northernmost part of the Golf of Bothnia, has hitherto been called *A. campestris* L. var. *bottnica* (LUNDSTR.) LINDB. FIL. This form, however, does not belong to *A. campestris* but to the arctic-circumpolar *A. borealis* PALL. and may for the present be called *A. borealis* var. *bottnica.*

In connection with my investigation of the taxonomic position of this form I was also faced the question of the chromosome numbers of *A. borealis, A. borealis* var. *bottnica* and *A. campestris.* I therefore procured seeds of *A. campestris* from botanic gardens and from wild individuals. The nothernmost of the spontaneous localities is Upsala, which is situated almost on the northern limit of the species. In order to make quite sure that the seeds from the botanic gardens belonged to the species in question, I cultivated them. *A. borealis* I cultivated from seeds of herbarium individuals. Living material of *A. borealis* var. *bottnica* I received from the original locality Pitsund at the estuary of the River Pite. To Mr. P. G. E. GUSTAFSSON, Piteå, who collected this material, I beg to express my sincere thanks.

The material is distributed as follows:

Artemisia borealis PALL. — Greenland: Regio Godthaabiana, in preta Ameralik (J. LAGERKRANTS, 1936).

Artemisia borealis var. *bottnica* (LUNDSTR.) LINDB. FIL. — Sweden: Pitsund, spontaneous on the bank of the River Pite (P. G. E. GUSTAFSSON, 1937).

Artemisia campestris L. — Germany: Bonn, the Botanic Garden, cult. (1937). Dresden, the Botanic Garden, cult. (1937). Frankfurt am Main, the Botanic Garden, cult. (1937). — France: Lyon, the Botanic Garden, cult. (1937). — Poland: Poznan, the Botanic Garden, cult. (1937). — Sweden: Scania, Vomb, spontaneous on sandy ground (J. LINDERS, 1937). Upsala, Kronåsen, spontaneous on sandy ground (S. ERLANDSSON, 1938).

The chromosome numbers were determined in root-tips, which had

been fixed in NAWASHIN's fixative according to MÜNTZING's modification. The thickness of the sections varied between 10—12 μ. The staining was carried out according to the method of FEULGEN (diamantfuchsin).

In pollen mother cells of *Artemisia maritima, cina, vulgaris, dracunculus, absinthium, campestris, annua* and *pontica,* WEINEDEL-LIEBAU (1928) determined $n = 9$ chromosomes. CHIARUGI (1927) found that the apomictic *A. nitida* has $2n = 27$.

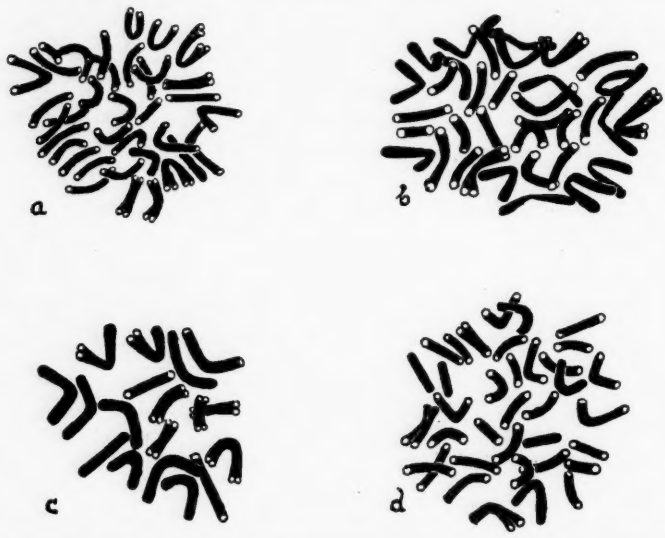

Fig. 1. Somatic metaphase plates of *Artemisia campestris a*: Sweden, Vomb ($2n = 36$); *b*: Germany, Bonn ($2n = 36$); *c*: *A. borealis* ($2n = 18$); *d*: *A. borealis* var. *bottnica* ($2n = 36$). — Magnif. 2700.

A. campestris. — My investigations of the chromosome numbers of this species showed $2n = 36$ throughout the whole material. This fact is astonishing as, according to the results of WEINEDEL-LIEBAU, one might have expected $2n = 18$. Thus we have here two different chromosome numbers, from which it is obvious that we have to reckon with at least two different races, one with the diploid chromosome number $2n = 18$ and the other tetraploid with $2n = 36$. It is to be regretted that WEINEDEL-LIEBAU does not indicate exactly the original locality of the *A. campestris* he investigated.

A. borealis. — The somatic number of this species was found to be

$2n = 18$. When comparing the sizes of the chromosomes in *A. borealis* and *A. campestris* I found that the chromosomes in *A. borealis* were considerably larger than in *A. campestris*.

In the outermost cell-layer of one root-tip of *A. borealis* I found three vertical, extremely larger cells, each of which included 36 chromosomes of the same size as in *A. campestris*.

A. borealis var. *bottnica*. — The chromosome number of this variety was found to be $2n = 36$. In spite of the similarity in appearances of this variety and *A. campestris* and the conformity as to the chromosome numbers, a close taxonomic investigation proves definitely that it is to be referred to *A. borealis*. As *A. borealis* is a very polymorphic species it is not surprising to find a deviating chromosome number in one of its varieties.

From a phytogeographical point of view this variety is of a certain interest. It must have immigrated to the coast of the Baltic Ice Lake when this lake was connected with the White Sea via the Kandalaksk Bay and the straits at Salla and Kitkajärvi in North Finland (HYYPPÄ, 1936). The individuals on the different localities, however, are of a uniform population, which fact is to be explained only by the circumstance that they originate from some few individuals of genetic similarity.

As this plant has immigrated into its present area of distribution after the last glaciation it may be a parallel to *Biscutella lævigata* L. in Central Europe (MANTON, 1934, 1937). Miss MANTON has shown that this species can be divided into three groups with reference to the chromosome numbers, with the numbers $2n = 18$, $2n = 36$ and $2n = 54$. The types belonging to the group $2n = 36$ occur in those parts of Central Europe which were glaciated during the last glaciation. The types belonging to this group are younger than those belonging to the one with 18 chromosomes, and are likely to have originated from some type belonging to the group $2n = 18$.

As things are at present, it is difficult to determine whether the origin of the *A. borealis* var. *bottnica* ($2n = 36$) from an *A. borealis* ($2n = 18$) is parallel to the origin of *Biscutella lævigata* types with 36 chromosomes. As has already been pointed out, *A. borealis* is a highly polymorphic species. There is also the possibility that the type now existing on the northern coast of the Golf of Bothnia is only an isolated population of a race that exists somewhere on the Russian-Siberian coast. When examining the *A. borealis* material of Riksmuseum, Stockholm, I found specimens from the Siberian coast, which were

extremely similar to *A. borealis* var. *bottnica*. Only when several types of *A. borealis* have been cytologically investigated will it be possible to determine the origin of *A. borealis* var. *bottnica*

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A GENERAL THEORY FOR THE INTERRELATION OF MEIOSIS AND MITOSIS

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(Preliminary note)

IN a series of papers the present author has published results, which in connection with the findings of other investigators seem to furnish an explanation of the interrelation of meiosis and mitosis, and also have some bearing on questions regarding reproduction of chromosomes, changes occurring in aged nuclei and how special groups of mutations arise. In a paper to be published shortly the author will give a summary of facts, obtained so far in this special and recently developed field of karyology. The starting point for the explanation of all these facts will be the hydration hypothesis put forward in a preliminary fashion in a paper on the mechanism of parthenogenesis (GUSTAFSSON, 1935) to cover the peculiar phenomena occurring in *Antennaria*, *Hieracium* and *Eupatorium* (the *Hieracium* type of parthenogenesis in the author's terminology or the *Eupatorium* type according to BERGMAN, 1935).

It can be proved that the origin or lack of meiotic divisions in EMC-nuclei of these three genera and some others does not depend on the duration of the premeiotic resting stage but on the degree of growth and sap intake in EMC:s and EMC-nuclei. An EMC-nucleus may either enter a sexual prophase with a subsequent reduction of the chromosome number, or a parthenogenetic meiotic prophase and metaphase without reduction in number, or, finally, a mitotic prophase and metaphase without any reduction. A certain minimum of growth and water intake is necessary in order to give the parthenogenetic type of development and another minimum is required in order to produce the mitotic division.

A somatic nucellus-cell, destined to develop aposporically and to form a gametophyte, cannot enter division immediately, because the formation of a gametophyte, even by means of mitotic divisions, is the result of a sexual tendency which must be overcome. The occurrence of a sexual tendency in purely somatic cells of aposporous genera is shown by the fact that in *Alchemilla* and *Leontodon* these cells can enter meiosis accidentally and even pass through the two meiotic

divisions. In order to form an aposporous gametophyte, they cannot counteract their sexual tendency in any other way than by increased growth and decreased viscosity, as shown above for the *Hieracium* type of parthenogenesis. In most aposporous genera this compromise between meiotic and mitotic influences has become stabilized.

A sexual meiotic nucleus does not show its strongest growth during the resting stage, but during the early prophase. An intense hydration of the nucleus takes place mainly after prophase has started, in fact at zygotene or early pachytene. The meiotic prophase nucleus increases about three or four times in size, exactly what has been recorded for the resting EMC-nuclei of *Hieracium* and other parthenogenetic genera.

Among the conclusions reached from these and other data regarding the physiology of meiosis, mitosis and their interrelation, the following may be mentioned:

a) Meiotically disposed nuclei can only overcome the sexual development by transferring the growth and hydration period from prophase to resting stage. Before a complete mitotic character is provided, a certain minimum of hydration is necessary.

b) Some special sexual influences may cause purely somatic cells to enter meiosis. In order to secure the formation of gametophytes in the case of aposporous development, primarily somatic but secondarily generative cells counteract the sexual tendency by a process identical with that occurring in EMC:s of *Antennaria* and *Hieracium*.

c) Reproduction of chromosomes does not take place until a certain hydration of the nucleus has occurred. Hydration and reproduction phenomena are accompanied or probably conditioned by changes of the water balance system of nuclei acids (HAMMARSTEN, 1924).

Palo Alto, Calif., September 1st, 1938.

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DIFFERENTIAL POLYPLOIDY WITHIN THE BLACKBERRIES

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SPECIES DIFFERENTIATION.

ALL European Rubi Eubati can be divided into two large groups: Eubati veri and Rubi Corylifolii. Eubati veri (as well as Rubi Corylifolii) consist of a series of species and microspecies with different taxonomic value. From a practical point of view they may be placed in six taxonomical groups. The first group comprises the very few main-species, which are sexual and diploid and have a southern area of distribution, namely *R. tomentosus* and *ulmifolius*. Both are plainly distinguished from other Rubi Eubati. The first-mentioned species forms a uniform and seemingly depauperated group of biotypes, while *R. ulmifolius* consists of a large number of forms closely related to each other. In spite of this polymorphy *R. ulmifolius* is a characteristic and isolated species. In the same group of species FOCKE [(1878 and) 1914] also placed *R. caucasicus* and *incanescens*, which so far have not been examined cytologically or genetically. Even *R. cæsius* — distributed throughout Europe and mostly rather common — is placed by him in this group. LIDFORSS (1914 and earlier) showed, however, that Scandinavian members of this species are obligate apomicts, and GUSTAFSSON (1933 a and unpublished; cf. also LONGLEY, 1924 and ROSANOVA, 1934) determined that several representatives have the tetraploid chromosome number.

Within the remaining Eubati veri there is a great variation due to hybridization. In fact, the different groups (subsectios) consist of a large number of transitional forms. Great many localized and endemic microspecies have arisen. Except a few species with a large area of distribution, the different regions display clusters of forms — often vegetatively propagated in a luxuriant manner — which it has been impossible to group together with the non-endemic species occurring in the region. But as has been shown by LIDFORSS, even several so-called good species are heterozygous to a surprisingly great extent. Crosses between them or even segregation products may therefore give origin to floras of blackberries, so rich in biotypes that each limited area con-

tains its own endemisms. This is especially true of the so-called Rubi Glandulosi, consisting of hundreds of microspecies grouped around *R. hirtus* and *R. serpens* (the pentaploid *R. Bellardii* also belongs to this group; GUSTAFSSON, 1933 b). It is as difficult to classify these microspecies as those of the Rubi Corylifolii. While these have their maximum of variation in northern Germany and southern Scandinavia, Rubi Glandulosi have a more southern type of distribution, with their greatest polymorphy in southern France, in Switzerland and Hungary. An astonishing fact is their pronounced capacity of migration into high-alpine areas (GUSTAFSSON, 1933 b).

Rubi Corylifolii are — as has been conclusively proved by LIDFORSS — primary and secondary hybrid and segregation products of crosses between Eubati veri and the tetraploid *R. cæsius*. In most cases it is impossible to elucidate with certainty which Eubati veri have taken part in these processes of hybridization. Probably in many cases, the Corylifolii occur in areas where the corresponding Eubati veri do not exist. Due to this panmixis a satisfactory phylogenetic and taxonomic treatment of the Corylifolii is impossible.

Rubi Eubati can be divided into the following six classes of varying taxonomical value.

I: Isolated, sexual species (cf. above).
II — VI: ± apomictic biotypes and biotype compounds.
II: Microspecies, which are taxonomically uniform and have a total-European area of distribution.
III: Microspecies, which form centres in larger complexes and have a large area of distribution.
IV: Microspecies, which are isolated and taxonomically distinguishable but have small areas of distribution.
V: Endemic microspecies, taxonomically isolated.
VI: Microspecies, which are localized and cannot be characterized taxonomically, mostly with transitional forms to other microspecies and compounds.

Eubati veri form part of the species groups I—VI, Rubi Corylifolii of the groups IV—VI.

There is no correlation between the taxonomical value of the microspecies and their chromosome numbers. Owing to the fact that polyploids with an odd number of genomes (3x, 5x) do not hybridize easily or in such a case give cytologically heterogeneous and non-viable offspring, a few of these polyploids tend to become isolated.

This grouping of Rubi Eubati into different classes according to their distribution, uniformity (phenotypical constancy) and systematical distinctness is rather arbitrary. Therefore a parallel to the different units of the sexual species (TURESSON, 1929 and earlier) cannot be drawn. The two sexual species, *R. tomentosus* and *R. ulmifolius*, are presumably two coenospecies. Most of the apomictic microspecies of groups II—VI (V) can, however, neither be equalled to coeno- or ecospecies, nor be regarded as ecotypes. Of special interest is the fact that the two microspecies, *R. thyrsanthus* and *candicans* (belonging to group IV) with the triploid chromosome number $3x = 21$, can be compared with climate ecotypes in the sexual species. Their areas of distribution do not agree. *R. thyrsanthus* has a more eastern, *R. candicans*, on the contrary, a more western distribution. Taxonomists have often regarded these two microspecies as hybrids of *R. tomentosus*. Their chromosome numbers favour this view ($n = 7 \times n = 14 \rightarrow 2n = 21$). The transitional forms in the boundary zone of the two areas are not necessarily crosses between the two microspecies (note the triploid chromosome number), but may be original hybridization products equal to the microspecies themselves.

As has been shown above, it is possible to search for single biotypes or biotype compounds in the great variety of hybrid and segregation products which keep isolated and for that reason can be treated systematically. However, all transitions in systematical value exist between these few microspecies, which can be classified without difficulty, and those which are groups of microspecies or groups of biotypes transitional to each other rather than different species. This fact renders the taxonomical treatment of the genus arbitrary and superficial. This has also been shown by the occurrence of the many types of monographs published. FOCKE, who studied the genus for about 50 years, finally adhered to a summary treatment. Microspecies, resembling each other, were placed together in groups, often in an artificial manner. Among the hundreds of microspecies, already described, most of those which were extremely localized, poorly known or badly described were left out, as well as most of the transitional forms connecting two or more microspecies.

So far as is known, apomixis occurs only in the subgenus Eubatus, but not in all groups. The South American representatives have not hitherto been examined experimentally, only one of them (*R. bogotensis*) cytologically. This species has the diploid chromosome number ($2n = 14$). The North American species, several of which are closely related

morphologically to the European Suberecti, are always sexual — with the exception of course of introduced species or hybrids of these (BRAINERD and PEITERSEN, 1921; GUSTAFSSON, 1930; DARROW, 1937). LONGLEY (1924) has examined many species and found polyploidy to be represented abundantly. In spite of their sexuality the North American species often form biotype compounds with transitions to each other which are difficult to classify.

THE DIFFERENT POLYPLOIDY WITHIN THE EUROPEAN BLACKBERRIES.

The facts mentioned above show that polyploidy is a common phenomenon among the Eubati veri. All apomictic microspecies are triploid, tetraploid or pentaploid. Of 39 examined microspecies from 46 different localities the number of representatives in the different groups of polyploidy is 3, 32 and 4 respectively. The tetraploids are plainly predominant (82 %). Hexaploids or microspecies with a still higher chromosome number do not occur, not even in the form spheres which are most heterogeneous and thus probably also most heterozygous. Nor do the endemic microspecies show a higher degree of polyploidy. Five out of 33 Scandinavian microspecies are endemic (*R. subvelutinus*, *R. confinis*, *R. tæniarum*, *R. vestervicensis*, *R. kollundicola*). The chromosome numbers are 21, 28, 28, 35, 35 respectively. Neither are the most northern representatives particularly high-polyploid. The most northern microspecies of the Swedish east coast and inland are *R. suberectus*, *R. sulcatus*, *R. plicatus*, and *R. thyrsanthus*, the first three being tetraploid, the last triploid. The most common microspecies along the west coast of Norway are *R. suberectus*, *R. plicatus*, *R. Selmeri*, and *R. fissus*, all tetraploid.

The few pentaploid microspecies are of particular interest. Two of them, *R. kollundicola* and *vestervicensis*, are localized microspecies. *R. cordifolius* was described from a small region. Later authors have widened its area of distribution but at the same time also changed the description. All are vegetatively luxuriant and apparently vital. Their small areas of distribution may therefore be attributed either to a late origin or to a lack of apomictic propagation (the first explanation, though not established, seems to be the more plausible one). *R. Bellardii*, on the other hand, is a powerful microspecies with a total-European area of distribution and a high constancy (cf. GUSTAFSSON, 1933 b).

Rubi Corylifolii have the chromosome numbers 28, 35, 42, ± 45,

49. Since 1933, 85 representatives have been examined and these correspond to ± 74 different types. In the different classes of polyploidy 2n = 28, 35, 42 and 45—49, respectively 42, 13, 17 and 2 types occur. Polyploids with a higher number than 28 are about as common as tetraploids. Corylifolii have therefore a greater tendency than Eubati veri to form high-polyploids. Only the chromosome number 28 implies an equilibrium in the Eubati veri, while among the Corylifolii the number 28 as well as 35 and 42 purport equilibria (57, 18 and 26 % respectively). The hexaploid Corylifolii occur exclusively in the western parts of the investigated region (cf. below). The western Corylifolii show the following numbers of types in the different classes: 2n = 28 (27 repr.), 2n = 35 (7 repr.), 2n = 42 (17 repr.), 2n = 45—49 (2 repr.). The percentages are 51, 13, 32 and 4. The surplus of tetraploid types has thus been still more diminished.

This difference in degree of polyploidy between the European Eubati veri and the Corylifolii cannot be the result of an unequally intense hybridization in the two groups. As mentioned above, Eubati veri form populations of hybrids and segregations, which in certain regions are as difficult to classify as the Corylifolii. Certainly, as yet only a few representatives belonging to Rubi Glandulosi have been examined cytologically (5 repr.) but all of them — except *R. Bellardii* — are tetraploid. According to MÜNTZING (1936) the varying viability in different classes of polyploidy is due to a chromosome optimum, above which the polyploid zygotes become non-viable or sublethal. The pentaploid Eubati veri are however — in spite of their rareness — vegetatively luxuriant and fertile. One of them, *R. Bellardii*, possesses a large area of distribution and probably a very great rate of spreading. Several plant genera are known in which polyploidy does not arise at all. There must be processes other than that mentioned above which prevent the origin of high-polyploidy or in certain plant genera even the occurrence of triploidy or tetraploidy. As the capacity to form restitution nuclei and unreduced gametes in certain parthenogenetic groups is genotypically determined (GUSTAFSSON, 1935 a), so the (pre)-meiotic formation or the postmeiotic function of unreduced gametes may depend upon the genotypical constitution. *R. Bellardii*, which has a disturbed meiosis, never forms dyads or triads. Pentads, hexads, heptads etc. occur, however, rather frequently. (Cf. also BLAKESLEE and BUCHHOLTZ, 1929 and SCHLÖSSER, 1936).

An interesting fact is that the American Eubati veri are not only total-sexual but that in spite of their sexuality they have a much

stronger tendency than the European apomicts to form high-polyploids. LONGLEY (1924) investigated 25 American and 3 European Eubati. Of the latter, *R. thyrsoideus* (a group name for *R. thyrsanthus* and related forms) had $2n = 21$, *R. corylifolius* and *R. cæsius* var. *turkestanicus* $2n = 28$. Of the 25 American Eubati veri 6 were diploid, 12 triploid, 2 pentaploid, 4 hexaploid, 1 octaploid. Apomixis and a high degree of polyploidy are therefore not necessarily simultaneous phenomena. The European Eubati veri must contain a physiological or genetical factor which prevents the increase of polyploidy or — from the other point of view — the American Eubati veri and in certain crosses *R. cæsius* must contain a character increasing polyploidy.

Very interesting in this connection is the spontaneous hybrid *R. idæus* × *cæsius*. It is highly sterile and lacks the capacity of apomictic propagation (pseudogamy a recessive phenomenon). Individuals, which appear morphologically to be back-crosses to *cæsius* and *idæus*, are not rare. From a theoretical point of view the primary hybrid should possess the triploid chromosome number ($n = 7 \times n = 14 \rightarrow \rightarrow 2n = 21$). This chromosome number explains the sterility in the absence of apomixis. Of 5 primary hybrids and back-crosses two possessed the triploid chromosome number (Rubi Scand. Chrom. Exam. Nos. 148 and 172), one the number 28 (147), one the number 35 (270) and one the number 42 (127). This hybrid has thus the capacity of forming polyploid biotypes in a high degree. ROSANOVA (1934) examined the artificial F_1-hybrids between *R. idæus* and *cæsius* and found in one offspring the number 28 (presumably $n = 14 \times n = 14$) and in one offspring the number 42 (presumably $n = 14 \times n = 28$). Most probably these data indicate the function of unreduced *R. cæsius*-gones. Why these do not function within the species itself or its crosses with certain Eubati veri and Corylifolii, cannot be answered. However, the lack of increase of polyploidy in the European Eubati veri does not depend upon a higher degree of fertility in the crosses between them for sterile hybrids (respectively back-crosses or segregations) are not infrequently produced in nature.

Similarly, among the spontaneous Corylifolii types occur which show a high degree of sterility. Each limited blackberry-flora contains a number of such products. One of the most interesting Scandinavian representatives in this respect is *R. glaucovirens*, which stands morphologically between *R. Wahlbergii* and *R. cæsius*. Its area of distribution is comparatively large: Blekinge, Bornholm, Skåne, Själland. In the east of Skåne and in Blekinge it is common. The chromosome number

is 4x = 28. Despite this balanced number it is highly sterile. The well-developed flower-stands carry only a few fruits and at most one or two ripe carpels per flower. Since *R. glaucovirens* is very uniform, its large area of distribution cannot be explained by the occurrence of parallel crossings in different parts of the area. Similar types with a higher chromosome number have not been found. All pentaploid biotypes of the area in question belong to quite different microspecies (mostly to *R. pruinosus*) and hexaploid biotypes have not arisen, either in the east of Skåne, Blekinge or Bornholm. The few seeds have probably without exception arisen apomictically, and their small number is sufficient to explain the great distribution of the microspecies.

THE DIFFERENTIAL POLYPLOIDY WITHIN THE CORYLIFOLII.

Although the number of examined Rubi Corylifolii amounts to only 100 representatives, and many gaps in our knowledge must be filled, definite conclusions can be drawn in certain respects.

Of 34 examined individuals from the eastern Rubus-area, representing ± 25 different types, 33 possessed the tetraploid or pentaploid chromosome number, none was hexaploid and one heptaploid. The equilibrium is undoubtedly 2n = 4x. Outside the *R. pruinosus*-complex only three representatives were pentaploid. Controls of the pentaploid number of *R. fioniæ* from Östergötland must be secured from new material. The two remaining types are localized (the pentaploid from Bornholm inadequately known). An interesting fact is that *R. pruinosus* always is pentaploid in the eastern as well as in the western Rubus-area in spite of its pronounced polymorphy. Taxonomists have often regarded *R. pruinosus* as a hybrid between *R. idæus* and Rubi Corylifolii. The pentaploid chromosome number can theoretically arise, if unreduced Corylifolii-gametes (4x = 28) are fertilized by or fertilize reduced *R. idæus*-gametes (x = 7). With this explanation several difficulties disappear. Apparently it is impossible to explain the polymorphy of *R. pruinosus* by the origin of mutations (embryo-mutations). Nor can segregations of *R. pruinosus* explain the great number of different types. Different chromosome numbers should then have arisen. Also the fact that (back) crosses between *R. idæus* and *R. cæsius* often resemble *R. pruinosus*, argues in favour of the opinion of taxonomists. Plant No. 127 in Rubi Scand. Chrom. Exam. has a strong resemblance to *R. pruinosus*. The hexaploid chromosome number distinguishes it

from all *R. pruinosus*-types. If this explanation is correct, unreduced gametes must now and then arise in certain crosses also among the east Swedish Corylifolii. Finally it may be mentioned that LIDFORSS in his experiments obtained types resembling *R. pruinosus* from the cross *R. thyrsanthus* × *R. cæsius.*

During the last years it has been shown that different chromosome races of a species (or species closely related to each other but with different chromosome numbers: ecospecies; TURESSON, 1929) have often very dissimilar distribution, ecology, life form (MÜNTZING, 1936; TISCHLER, 1935). The changed properties, brought about by the increased chromosome numbers (often through autopolyploidy) show themselves to be mainly quantitative — in size, winter-hardiness, amount of vitamins, and so on. These changed properties permit the coenospecies to widen its area of distribution. The environment does not change the chromosome numbers (HAGERUP, 1931) but acts only passively through selection among biotypes with different chromosome numbers.

Even purely historical factors and factors due to immigration phenomena must be of very great importance. This is completely proved by the difference with regard to degree of polyploidy in the subatlantic and the middle Baltic areas. (The differential polyploidy may even serve as a factor separating these two plant-geographical regions; GUSTAFSSON, 1935 b). For nobody can maintain that the subatlantic or atlantic climates are able to produce high-polyploids by themselves, while the middle Baltic climate lacks this ability. Nor is there any reason to assume that high-polyploids grow better in the western than in the eastern area. The cause of the differential polyploidy must lie, therefore, in the fact that the immigrants to the eastern area consisted and still consist of biotypes and biotype compounds lacking the power of increasing the chromosome number above the pentaploid status. The immigrants to the western area, on the other hand, possessed this capacity.

Several microspecies show different chromosome numbers in the eastern and in the western areas. This is especially true of *R. Wahlbergii* and *R. ambifarius* (coll.). Seven representatives of the first microspecies have been examined (in the investigations before 1933 also two other individuals). The chromosome numbers are the following: East-Sweden 28, ± 30, (28), West-Sweden + Norway 28—29, 35, 35, 35 (42), Denmark 35. The related species, *R. vexatus*, has 6x = 42. *R. ambifarius* gives for the two areas respectively 28, (28), and 28, 35, 42.

R. Mortensenii, growing in the western region, shows the numbers 28, 27—28, 28, 42, *R. eluxatus*, also in the western region, 28, and in an earlier investigation ± 45. An individual phenotypically related to *R. eluxatus* (Rubi Scand. Chrom. Exam. No. 175) revealed also the number ± 45. *R. leiocarpus* gave the numbers 28, 49. *R. nitens* behaves in the same way, possibly also *R. ciliatus* and *R. tiliaceus*. *R. nitens* is a polymorphous microspecies. The existence of two different chromosome races (28, 42) is therefore not difficult to interpret. Against these numerous cases of chromosome races in the western region only one sure case from the eastern region can be mentioned: two *R. gothicus*-types from Bornholm have $2n = 28$ and 35. Chromosome races containing the hexaploid numbers have never arisen in the eastern region.

These cases of differential polyploidy within a microspecies prove beyond doubt the view that in the western area phenomena exist which produce high-polyploids and change low-polyploids, immigrated from other regions, into high-polyploids. Similar Corylifolii may of course have arisen from different crosses, so that the eastern tetraploids are not necessarily the ancestors of the resembling western penta- and hexaploids but only parallel products. The western Corylifolii cannot possibly have arisen from the eastern biotypes exclusively through autopolyploidy, because autopolyploids would then have arisen in the eastern region itself. Since the representatives of a microspecies, containing different chromosome races, are not merely superficial in resemblance but are almost identical phenotypically (*R. Wahlbergii*, partly *R. ambifarius*), affinity in a phylogenetical sense is probable. This is obvious when high- and low-chromosomal types occur in the same region (*R. leiocarpus*, *R. Mortensenii*, *R. nitens*, partly *R. Wahlbergii*).

SUMMARY.

1. European apomictic Eubati veri are triploid, tetraploid or pentaploid but never hexaploid or heptaploid. 82 % of the apomictic microspecies are tetraploid.

2. American Eubati veri (sexual) are diploid, triploid, pentaploid, hexaploid and octaploid.

3. *Rubus idæus* × *cæsius* easily forms unreduced gametes. Therefore the F_1 and back-crosses exhibit the triploid, tetraploid, pentaploid and hexaploid chromosome numbers. *R. cæsius* is capable of forming unreduced gametes as father-parent in the cross *R. idæus* × *cæsius*.

Nevertheless, so far as is known, *R. cæsius* itself is always tetraploid in nature.

4. East-Scandinavian Rubi Corylifolii are mostly tetraploid. No hexaploids occur. Most pentaploids belong to *R. pruinosus* coll.

5. West-Scandinavian Rubi Corylifolii are tetraploid, pentaploid, hexaploid, superhexaploid, heptaploid.

6. Several eastern Corylifolii increase their chromosome numbers when migrated to the western region. Several western Corylifolii contain different chromosome races, often with the tetraploid or pentaploid + the hexaploid or heptaploid number.

7. The differential polyploidy cannot be due to a different degree of heterozygoty or sterility, nor to a selectional effect of climate. Neither is there any possibility of the subatlantic climate directly producing polyploids with high chromosome numbers.

8. The differential polyploidy within the Corylifolii is probably due to an increase of the chromosome number in crosses between *R. cæsius* and atlantic Eubati veri or to crosses between atlantic Corylifolii (which themselves have arisen from crosses between *R. cæsius* and Eubati veri) and to status quo in crosses between *R. cæsius* and eastern Eubati veri or between eastern Corylifolii.

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EUROPEAN EUBATI VERI.

Rubi Scandinavici, chromosomi examinati.

The author began some years ago to collect material for a parallel cytological and taxonomical investigation of the genus *Rubus*. This exsiccate is made in cooperation with Mr. C. E. GUSTAFSSON, Trelleborg, Sweden, and when finished will be deposited in the Botanical Museums of Lund and Stockholm.

Triploid species.

$2n = 3x = 21$.

R. thyrsanthus F. (No. 40)
R. subvelutinus LBG. (No. 120)

Tetraploid species.

$2n = 4x = 28$.

R. fissus LINDL. (No. 189)
R. Scheutzii LBG. (No. 116)
R. confinis LBG. (No. 51)

R. Langei JENS. (No. 250)
R. Selmeri LBG. (Nos. 190, 193)
R. cimbricus F. (No. 69)
R. egregius F. (No. 181)
R. Gelerti FRID. (No. 234)
R. scanicus ARESCH. (No. 53)
R. sciaphilus LGE (Nos. 78, 263)
R. radula WHE et N. (No. 218)
R. tæniarum LBG. (No. 47)
R. pallidus WHE et N. (No. 221)
R. atrichantherus KRAUSE grex *mucronatus* (No. 75)
R. Drejeri JENS. (Nos. 82, 235)

Pentaploid species.

2n= 5x = 35.

R. kollundicola GUST. (No. 65)
R. Bellardii WHE et N. (No. 62)

Chromosome numbers of other Eubati veri, examined by the author.

Diploid species.

R. tomentosus BORKH.
R. ulmifolius SCOTT.

Triploid species.

R. candicans WHE et N.

Tetraploid species.

R. suberectus ANDS.
R. plicatus WHE et N.
R. affinis WHE et N.
R. insularis ARESCH.
R. polyanthemus LINDEB.
R. Sprengelii WHE et N.
R. Arrhenii LGE
R. Genewierii MÜLL.
R. cletrophilus MÜLL.
R. pallidus WHE et N. (f. *suecicus*)
R. radula WHE et N. v. *angustifolius* LUND
R. Kaltenbachii METCH.
R. hirtus W. et K.
R. pubescens WHE et N.
R. dasyphyllus ROG.
R. vestitus WHE et N.
R. rudis WHE et N.
R. Lindebergii MÜLL.

Pentaploid species.

R. vestervicensis GUST.
R. cordifolius WHE et N.
(*R. Bellardii* WHE et N., two specimens)

R. cæsius L. (Different representatives examined. Rubi Scand. Chrom. Exam. No. 244). Always $2n = 4x = 28$.
Spontaneous *R. idæus* × *cæsius* and back-crosses.
R. idæus × *cæsius*: 1. $2n = 3x = 21$ (Nos. 148, 172)
2. $2n = 4x = 28$ (No. 147)
3. $2n = 5x = 35$ (No. 270)
4. $2n = 6x = 42$ (No. 127)

RUBI CORYLIFOLII.

I. *The East of Sweden.*

1. Blekinge—Uppland.

	No.	
R. Wahlbergii ARRH.	151	$2n = 4x = \pm 30$
R. Wahlbergii ARRH. v. *tenuifolius* ARESCH.	4	28
R. nemorosus ARRH. v. *suberiocarpus* ad int.	86	28
R. nemorosus ARRH. v. *subglandulosus* LUND	115	28
R. gothicus FRIDER. p. p.	94	28
R. centiformis FRIDER. v. *Lidforssii* (GEL.)	2	28—35
R. centiformis FRIDER. v. *Lidforssii* (GEL.) forma	136	28
R. ambifarius MÜLL.	137	28
R. fioniæ FRIDER.	3	28
R. fioniæ FRIDER.	133	28
R. fioniæ FRIDER.	153	28
R. fioniæ FRIDER.	12	28—29
R. ciliatus LINDEB.	1	28
R. ciliatus LINDEB.	145	± 30
R. »(*cæsius* × *Wahlbergii*)» f. *glaucoformis* GUST.	154	28
R. pruinosus ARRH.	110	$2n = 5x = 35$
R. pruinosus ARRH.	111	35
R. pruinosus ARRH.	143	35
R. pruinosus ARRH.	149	± 35
R. »*cæsius* × *fioniæ*»	121	35
Unknown or undetermined	132	$2n = 4x = 28$
» » »	155	28—29

In addition the chromosome numbers of different other representatives of *R. Wahlbergii, nemorosus, gothicus* from Småland have been examined. In all cases $2n = 4x = 28$ was obtained. *R. balticus* ARESCH. from Småland has $2n = 7x = 49$, *R. fioniæ* from Östergötland has $2n = 5x = 35$, *R. ambifarius* (cultivated in Lund Bot. Garden) has $2n = 4x = 28$, and finally two different forms of *R. gothicus* from the island of Bornholm have $2n = 4x = 28$ and $2n = 5x = 35$.

2. The East of Skåne.

	No.	
R. gothicus FRIDER. p. p. f. *microphylla*	165	$2n = 4x = 28$
R. gothicus FRIDER. p. p.	168	± 28
R. pruinosus ARRH. v. *silvaticus* ad int.	157	$2n = 5x = \pm 35$
R. ruderalis ARESCH.	185	$2n = 4x = \pm 30$

II. *The West of Sweden* (*Norway and Denmark*).

1. The West of Skåne.

	No.	
R. Wahlbergii ARRH. f. *umbrosa*	246	2n = 5x = 35
R. centiformis FRIDER. v. *Mortensenii* FRIDER. et GEL.	243	2n = 4x = 28
R. centiformis FRIDER. v. *Mortensenii* FRIDER. et GEL.	239	2n = 6x = 42
R. centiformis FRIDER. v. *Mortensenii* FRIDER. et GEL.	179	2n = 4x = 27—28
R. »*cæsius* × *Mortensenii*»	188	28
R. ciliatus LINDEB. forma	174	28
R. ciliatus LINDEB. v. *tiliaceus* (ARESCH.)	59	28
R. »*ciliatus* × *eluxatus*»	175	2n = ± 45
R. »(*cæsius* × *ciliatus*)» f. *progenerans* LIDF.	180	2n = 6x = 42
R. eluxatus NEUM. ad subnit. vergens	178	2n = 4x = ± 28
R. »*cæsius* × *gothicus*» f. *ruedensis* (LIDF.)	245	2n = 6x = 42
Unknown	177	42

In 1933 another list of chromosome numbers was published. The chromosome numbers of *R. Wahlbergii* from Söderåsen, *ciliatus* and *tiliaceus* from Kullen were listed as hexaploid, and the numbers of *R.* »*trivultus* f. *Kullensis*» (related to *R. ambifarius* MÜLL.?) and of *R. eluxatus* NEUM.* *subnitidus* LIDF. as tetraploid and superhexaploid respectively. Since that year two other biotypes have been examined (*R. cyclophyllus* LINDEB.? and *R. permixtus* ARESCH., the latter cultivated in Lund Bot. Garden but both originating from Kullen). The chromosome numbers were 6x = 42 and 4x = 28 respectively.

2. Halland, Bohuslän (Norway).

	No.	
R. dissimulans LINDEB. p. p. v. *suberectiformis*	209	2n = 4x = 28
R. hallandicus (GABR.) NEUM.	215	28
R. Wahlbergii ARRH. forma	39	28—29
R. Wahlbergii ARRH.	25	2n = 5x = 35
R. Wahlbergii ARRH. v. *partitus* GUST.	201	35
R. (*cæsius* × *gothicus*) f. *acutus* (LINDEB.)	210	2n = 6x = 42
R. rosanthus LINDEB. v. *leiocarpus* LINDEB.	43	2n = 4x = 28
R. rosanthus LINDEB. v. *leiocarpus* LINDEB.	27	2n = 7x = 49
R. rosanthus LINDEB. v. *eriocarpus* LINDEB.	41	2n = 5x = 35
R. cyclophyllus LINDEB.	42	2n = 6x = 42
R. Olavii NEUM. f.	28	± 42
Unknown	48	2n = 4x = 28

In 1933 another representative of *R. dissimulans* (*R. nitens*), cultivated in Lund Bot. Garden, but originating from Bohuslän, was examined (6x = 42).

3. Denmark.

	No.	
R. dissimulans LINDEB. v. *selectus*	253	2n = 6x = 42
R. serrulatus LINDEB. f. *ditior* FRIDER.	266	2n = 4x = 28
R. Wahlbergii ARRH. v. *magnificus* FRIDER.	269	2n = 5x = 35
R. vexatus FRIDER. v. *crispus* FRIDER.	247	2n = 6x = 42

	No.	
R. gothicus FRIDER.	66	2n = 4x = 28
R. gothicus FRIDER.	72	28
R. gothicus FRIDER.	262	28
R. gothicus FRIDER.	264	28
R. gothicus FRIDER.	268	28
R. centiformis FRIDER. p. p.	225	28
R. centiformis FRIDER. p. p.	251	28
R. ambifarius MÜLL.	249	28
R. ambifarius MÜLL. f.	184	2n = 5x = 35
R. fasciculatus MÜLL. = *R. ambifarius* MÜLL.?	274	2n = 6x = 42
R. fioniæ FRIDER.	275	2n = 4x = 28
R. ferox FRIDER.	258	2n = 6x = ± 42
R. ferox FRIDER.	260	± 42
R. Ostenfeldii FRIDER. v. *micrander* FRIDER.	273	2n = 4x = 28
R. Ostenfeldii FRIDER. v. versus *Warmingii*	223	2n = 6x = 42
R. Ostenfeldii FRIDER. v. versus *Warmingii*	271	2n = 6x = ± 44
R. pruinosus ARRH. v. *Warmingii* (JENS.)	265	2n = 5x = 35
R. phylloglotta FRIDER.	277	2n = 4x = 28
R. hystricopsis FRIDER.	267	28
R. »(*cæsius* × *Drejeri*)» v. *aberrans* (FRIDER.)	252	28
R. »*cæsius* × *Lindebergii*»	248	2n = 6x = 42
R. »*cæsius* × *vestitus*»	84	2n = 4x = 28
R. »*cæsius* × *vestitus*»	279	28
Unknown or undetermined	280	28
» » »	68	28
» » »	77	28
» » »	276	28
» » »	255	± 30
» » »	256	2n = 6x = 42
» » »	257	42
» » »	254	± 44

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ERBLICHKEITSVERSUCHE MIT PISUM

X. DIE KOPPELUNGSGRUPPEN Pa-R-Tl-Btb UND Wlo-P-Pl

VON *ERNST NILSSON*

HAMMENHÖG, SCHWEDEN

EINLEITUNG.

IM Jahre 1930 begann ich eine Untersuchung um festzustellen, ob die von KAPPERT (1925) entdeckte Koppelung zwischen den Genen für glatte Samen (*R*) und spitze Hülse auf *R—Btb* zu beziehen ist. RASMUSSON (1927) hat nämlich zwei verschiedene Gene, *Bta* und *Btb*, für stumpfes Hülsenende veröffentlicht, und selbst glaubte ich in für praktische Zwecke ausgeführten Kreuzungen einen Zusammenhang zwischen *R* und *Btb* zu finden. Später wurden auch andere Gene in diese Koppelungsuntersuchung einbezogen, die mit den bisher erhaltenen Resultaten zeigen, dass die Koppelungsgruppen 3 und 7 von WINGE (1936) teilweise zu einer und derselben Gruppe gehören.

Der zweite Teil dieser Untersuchung behandelt den Zusammenhang zwischen dem von mir (ERNST NILSSON, 1933 b) entdeckten Gen *Wlo* und anderen Genen. Es ist hierbei gelungen *Wlo* in einer schon früher bekannten Koppelungsgruppe unterzubringen, was in der zitierten Arbeit schon kurz erwähnt worden ist.

Die monohybriden Spaltungen sind in der bisher gebräuchlichsten Weise (laut JOHANNSEN, 1926) berechnet worden. Dihybride Spaltungen sind mit dem χ^2-Wert (FISHER, 1936) beurteilt, nachdem sie zuerst laut FISHERS »test of independence» (l. c. pag. 89) berechnet worden sind, der mit der von KAPPERT (1927) beschriebenen Methode für die Beurteilung dihybrider Zahlen übereinstimmt. In Fällen von Koppelung ist der Wert für das Crossingover laut FISHERS (l. c.) T_4-Methode berechnet worden [nach der Gleichung $n\Theta^2 - (a - 2b - - 2c - d)\,\Theta = 2\,d$].

DIE GRUPPE Pa-R-Tl-Btb.

LITERATURÜBERSICHT.

Dass *R* und *Tl* sehr stark gekoppelt sind, ist durch mehrere Untersuchungen schon seit 1911 (VILMORIN and BATESON, übrige Lit. siehe

WINGE) bekannt. KAPPERT (1925) hat Koppelung zwischen *R* und *Bt* nachgewiesen (laut vorliegender Studie *Btb*, was nebenbei auch von ERNST NILSSON, 1933 a, S. 203, erwähnt worden ist). Auch PELLEW and RICHARDSON SANSOME (1931) haben ohne Zahlen anzuführen mitgeteilt, dass *R* und *Bt* gekoppelt sind, während RASMUSSON (l. c.) keine Koppelung zwischen *R* und einem der *Bt*-Gene gefunden hat. WINGE (l. c.) hat *R* und *Tl* in seine Koppelungsgruppe 7 untergebracht, während *Pa* und *Btb* in Gruppe 3 eingereiht wurden. WINGE erachtet es nämlich als unsicher, ob zwischen *R* und einem der Gene für stumpfes Hülsenende Koppelung besteht, oder ob der von KAPPERT (1925) zuerst nachgewiesene Zusammenhang eine Folge von Translokation ist.

Unten folgt als Zusammenfassung eine Übersicht über die möglichen Relationen zwischen den hier in Rede stehenden Allelpaaren und den Arbeiten, in denen sie studiert worden sind.

Pa—R Diese Arbeit.
Pa—Tl Diese Arbeit.
Pa—Btb Diese Arbeit.
R—Tl VILMORIN and BATESON (1911) u. a. m.
R—Btb KAPPERT (1925), RASMUSSON (1927), PELLEW and RICHARDSON SANSOME (1931) und diese Arbeit.
Tl—Btb Diese Arbeit.

In diesem Zusammenhang sollen auch die Beziehungen zwischen *Pa* und *Btb* sowie den übrigen Genen kurz behandelt werden, die von WINGE (l. c.) zur Koppelungsgruppe 3 gerechnet werden, da diese Frage im Zusammenhang mit den Versuchsresultaten nicht erörtert werden. Zur Gruppe 3 führt WINGE die Gene *N—Ss—Q—Pa—Btb*. Das Gen *Ss* geht häufig unter der Bezeichnung *Cp*, das zum erstenmal von WELLENSIEK (1925 a) verwendet worden ist. In einer späteren Arbeit im gleichen Jahr hat WELLENSIEK (1925 c, S. 359) indessen hervorgehoben, dass die Bezeichnung *Ss* — etwas früher von WHITE benutzt — Prioritätsrecht hat. *Q* ist von WELLENSIEK and KEIJSER (1929) als Bezeichnung für ein dominantes Gen eingeführt worden, das erblichen Abort bedingt.

Die Eigenschaft gerade—gekrümmte Hülse, für die die Bezeichnung *Ss* (bzw. *Cp*) eingeführt worden ist, ist eine deutlich quantitative Eigenschaft. Wenn man eine zahlenmässige Bezeichnung für den Krümmungsgrad, z. B. meinen Krümmungsindex (ERNST NILSSON, 1929), von in dieser Eigenschaft spaltenden Nachkommen benutzt, wird man finden, dass eine markierte Grenze zwischen geraden und ge-

krümmten Hülsen wenigstens nicht immer festzustellen ist. Die Grenze zwischen der Dominanz- und Rezessivklasse wird dann auch willkürlich. RASMUSSON (1927) hat auch betont, dass die Krümmung der Hülse als eine polymere Eigenschaft zu betrachten ist. Dies hindert natürlich nicht, dass man einen Zusammenhang zwischen Krümmung der Hülse und Eigenschaften, die durch bestimmte Gene bedingt sind, nachweisen kann, und dass solche Ergebnisse für die Unterbringung von Genen in Koppelungsgruppen wegweisend sein können. Dagegen dürfte man auf Grund solcher Untersuchungen kaum einen sicheren Wert für das Crossingover bestimmen können oder berechtigt sein der genetischen Unterlage für die Krümmung der Hülse einen bestimmten Platz in der Koppelungsgruppe anzuweisen.

WELLENSIEK (1925, 1928) hat einen starken Zusammenhang zwischen *N* und der Krümmung der Hülse konstatiert und basiert hierauf einen Crossingover von 15 % berechnet. Dass der Zusammenhang, der zweifellos zwischen Wanddicke und Krümmung der Hülse besteht, auf Koppelung zwischen *N* und einem Gen für die Hülsenform und nicht auf pleiotroper Wirkung von *N* beruht (siehe ERNST NILSSON, 1929) dürfte unmöglich zu beweisen sein, solange keine Kreuzung der Form *N ss* × *n Ss* untersucht ist. Ein deutlicher Zusammenhang zwischen Krümmung der Hülse und *Btb* ist von WELLENSIEK (1925 b), RASMUSSON (l. c.) und in mehreren meiner Kreuzungen festgestellt worden. Desgleichen hat DE WINTON (siehe WINGE l. c.) einen Zusammenhang zwischen Krümmung der Hülse und *Pa* (ein Gen für dunkelgrüne kontra hellgrüne Hülsenfarbe, nicht zu verwechseln mit *Gp* und *O*) festgestellt. Dies hatte zur Folge, dass die Gene *Pa* und *Btb* beide in WINGES Koppelungsgruppe 3 untergebracht wurden. Der Zusammenhang zwischen einerseits Krümmung der Hülse und andererseits den Genen *Pa* und *Btb* beruht mit Sicherheit auf Koppelung, da, wie aus vorliegender Arbeit (siehe unten) hervorgeht, *Pa* und *Btb* mit Sicherheit zur gleichen Koppelungsgruppe gehören.

Ob *Q* wirklich ein dominantes Gen in einem Allelenpaar ist, oder ob der erbliche Abort, der laut WELLENSIEK and KEIJSER (1929) durch *Q* bedingt wird, die Folge einer reziproken Translokation zwischen nicht homologen Chromosomen ist, kann auf Grund bisheriger Untersuchungen kaum mit Sicherheit entschieden werden. WELLENSIEK and KEIJSER (1929) und WELLENSIEK (1929 a) haben gefunden, dass die Eigenschaften Abort und gekrümmte Hülsen abhängig voneinander vererbt werden, was auch für die Eigenschaften dicke Hülsenwand (*n*) und Abort gilt. Ein Crossingover kommt für beide Interrelationen etwa

gleich häufig vor. Die Beziehung zwischen *N* und *Ss* (siehe oben) ist sehr variabel, hat aber im Durchschnitt ungefähr gleichen Wert wie für *Q—Ss* und *Q—N*. WINGE (l. c.) bringt diese Gene in folgende Reihenfolge (Fig. 1), aber es besteht kein Hindernis sie an Hand der Resultate in eine beliebige andere Reihenfolge zu bringen.

Die gefundenen relativen Abstände zwischen *N—Ss—Q*, die sehr schlecht mit der Theorie über die lineare Anordnung der Gene übereinstimmen, können sehr gut mit dieser in Einklang gebracht werden, wenn man den Zusammenhang zwischen *N* und *Ss* als einen pleiotropen Effekt von *N* auffasst. Beruht der Abort, der mit *Q* symbolisiert wird, auf reziproker Translokation und ist demnach identisch mit der Erscheinung, die als Semisterilität bezeichnet wird, so stehen die gefundenen Resultate auch mit der genannten Theorie nicht im Widerspruch, ob nun der Zusammenhang zwischen *N* und *Ss* auf wirkliche Koppelung oder Pleiotropie beruht.

Fig. 1. WINGES Koppelungsgruppe 3 mit Ausnahme der Gene *Pa—Btb* (Literatur, siehe WINGE, 1936).

Ein direkter Zusammenhang zwischen *N* und *Pa—Btb* hat nicht festgestellt werden können, und meiner Ansicht nach ist es sehr zweifelhaft, ob man mit einer quantitativen Eigenschaft wie die Krümmung der Hülse als Verbindungsglied *N* als zur gleichen Koppelungsgruppe wie *Pa—Btb* rechnen kann.

STUDIERTE GENE UND KREUZUNGEN.

Folgende Linien sind als Kreuzungsmaterial verwendet worden. Nach der Nummer der Linie und dem Namen der Sorte, aus der diese stammt, ist im Verzeichnis ihr Genotypus mit Rücksicht auf die hier behandelten Gene angegeben, soweit diese in Kreuzungen mit den betr. Linien als Eltern studiert worden sind.

L. 8	Witham Wonder	*r, Tl, btb*
L. 11	Lincoln	*r, btb*
L. 31	Non pareil (Linie nicht typisch für die Sorte)	*pa, r, Btb*
L. 34	Extra Rapid	*R, Btb*
L. 36	Snabel	*Pa, R, btb*

L. 38 Sabre	*R, Btb*
L. 40 »Kungshöga»	*pa, Tl, Btb*
L. 54 Acacia	*tl, Btb*
L. 64 Goldkönig	*r, btb*
L. 77 Bohnenerbse	*R, Btb*
L. 85 Chenille-Typus (*s*)	*R, Btb*
L. 86 »Pisum jomardi»	*R, Btb*
L. 89 *wlo*-Linie aus English Wonder	*r, Btb*
L. 92 Meteor	*R, btb*
L. 94 Rekord	*R, btb*
L. 96 *fl*-Linie aus Onward	*pa, Tl, Btb*
L. 97 Rogues-Typus	*R, btb*
L. 114 Purpurhülsig	*R, Btb*
L. 121 Ambrosia	*Pa, Btb*
L. 129	*tl, Pa*
L. 144 Primärtypus F (ERNST NILSSON)	*R, Btb*
L. 145	*Pa, tl, btb*
L. 146	*pa, Tl*
L. 147	*Pa, r*
L. 148	*pa, R, btb*

Folgende Kreuzungen sind bearbeitet worden (reziproke Kreuzungen mit gleicher Nummer).

Kreuzung				
I.	L. 114 × L. 8	*R Btb*	×	*r btb*
II.	L. 86 × »		×	»
III.	L. 77 × »		×	»
IV.	L. 86 × L. 64		×	»
V.	L. 85 × »		×	»
VI.	L. 77 × »		×	»
VII.	L. 38 × L. 11		×	»
VIII.	L. 144 × » (F_3)		×	»
IX.	L. 8 × L. 34		×	»
X.	L. 97 × L. 89	*R btb*	×	*r Btb*
XI.	L. 89 × L. 92		×	»
XII.	» × L. 94		×	»
XIII.	L. 36 × L. 31	*Pa R btb*	×	*pa r Btb*
XIV.	L. 8 × L. 54	*Tl btb*	×	*tl Btb*
XV.	L. 96 × L. 145	*pa Tl Btb*	×	*Pa tl btb*
XVI.	L. 145 × L. 40	*Pa tl btb*	×	*pa Tl Btb*
XVII.	L. 146 × L. 129	*pa Tl*	×	*Pa tl*

XVIII. L. 147 × L. 148 *Pa r* × *pa R*
XIX. L. 148 × L. 121 *pa btb* × *Pa Btb*

Der Effekt der in Rede stehenden Gene braucht hier nicht näher erörtert zu werden, da sämtliche ausser *Pa* seit langem bekannt und wiederholt Gegenstand von Untersuchungen gewesen sind. Dem Allelen-

TABELLE 1. *Monohybride Spaltungen in Pa, R, Tl und Btb.*

Kreuzung	F_1	n	Anzahl Rezessive pro 4	D:m_k
I	*Rr*	282	1,09	0,9
II	»	702	0,94	0,9
III	»	504	0,76	3,1
IV	»	467	1,13	1,6
V	»	190	1,39	3,1
VI	»	281	0,88	1,2
VII	»	292	0,86	1,4
VIII	»	82	0,63	1,9
IX	»	90	0,98	0,1
X	»	117	1,13	0,8
XI	»	250	1,17	1,6
XII	»	164	0,90	0,7
XIII	»	712	1,02	0,3
XVIII	»	1075	0,87	2,5
Σ	»	5208	0,96	1,7

Kreuzung	F_1	n	Anzahl Rezessive pro 4	D:m_k
I	*Btb btb*	282	1,05	0,5
II	»	702	1,09	1,4
III	»	504	1,16	2,1
IV	»	467	0,96	0,5
V	»	190	0,97	0,2
VI	»	281	0,78	2,2
VII	»	292	1,00	0
VIII	»	82	1,02	0,1
IX	»	90	1,05	0,5
X	»	117	0,82	1,1
XI	»	250	0,74	2,4
XII	»	164	0,90	0,7
XIII	»	712	1,14	2,2
XIV	»	272	1,26	2,5
XV	»	158	0,84	1,2
XVI	»	149	1,05	0,4
XIX	»	1607	0,96	0,9
Σ	»	6319	1,01	0,5

Kreuzung	F_1	n	Anzahl Rezessive pro 4	D:m_k
XIII	*Pa pa*	712	1,02	0,3
XV	»	158	1,09	0,7
XVI	»	149	1,02	0,1
XVII	»	261	0,90	0,6
XVIII	»	1075	0,97	0,6
XIX	»	1607	0,74	6,0
Σ	»	3962	0,89	4,0
XIV	*Tl tl*	272	0,93	0,7
XV	»	158	0,78	1,6
XVI	»	149	0,67	2,3
XVII	»	261	0,92	0,8
Σ	»	840	0,85	2,5

paar *R—r* entspricht die Eigenschaftsdifferenz runde—runzelige (Markerbsen-) Samen, *Btb—btb* der Differenz stumpfes—spitzes Hülsenende und *Tl—tl* der Differenz Blätter mit Ranken—Blätter mit Fiederblättchen anstatt Ranken (sog. Acacia-Blätter). *Pa—pa* ist ein Genpaar, dessen rezessives Allel eine dunkelgrüne Hülse bedingt, während die dominante Form eine hellere grüne Hülsenfarbe verursacht. Dieser Unterschied kann in gewissen Kreuzungen scharf markiert sein, sodass die Klassifikation keinerlei Schwierigkeiten verursacht, während in anderen Spaltungen die Grenze zwischen den beiden Klassen etwas unsicher ist. Offenbar wird der Effekt von *Pa* durch die Wirkung anderer Gene beeinflusst. Es sei hervorgehoben, dass *Pa* nicht mit *O* und *Gp* zu verwechseln ist, die in ihren *rezessiven* Formen mehr oder

weniger gelbe Hülsen bedingen, während es hier das *dominante* Gen ist, das blassgrüne Hülsen gibt. Die Beurteilung der Eigenschaften stumpfe—spitze Hülse ist stets im grünen Zustand erfolgt, weshalb ein Zweifel hinsichtlich der Klassifikation nicht in Frage kommen kann.

In Tabelle 1 sind die wichtigsten Daten in bezug auf die monohybriden Spaltungen angeführt. Sie dürften keine besondere Be-

TABELLE 2. *Dihybride Spaltungen in R—Btb.*

Kreuzung		n	Gefundene Anzahl				Differenz für *aabb* (gefunden-erwartet)	P	Crossing-over
Nr.	Typus		*R Btb*	*R btb btb*	*rr Btb*	*rr btb btb*			
I	*R Btb* × *r btb*	282	158	47	50	27	+ 5,8	0,1—0,05	41,7
II	»	702	419	119	92	72	+ 27,4	< 0,01	36,6
III	»	504	309	99	49	47	+ 19,2	»	34,4
IV	»	467	273	62	82	50	+ 18,4	»	37,0
V	»	190	105	19	39	27	+ 11,0	»	33,5
VI	»	281	181	38	45	17	+ 4,9	0,1—0,05	41,3
VII	»	292	185	44	34	29	+ 13,2	< 0,01	33,3
VIII	»	82	55	14	6	7	+ 3,7	0,02— 0,01	32,0
IX	»	90	57	11	10	12	+ 6,4	< 0,01	26,9
Σ	»	2890	1742	453	407	288	+ 109,8	< 0,01	36,6
X	*R btb* × *r Btb*	117	62	22	31	2	— 4,8	0,02—0,01	26,7
XI	»	250	140	37	64	9	— 4,4	0,2—0,1	40,6
XII	»	164	97	30	30	7	— 1,3	0,7—0,5	46,3
XIII	»	712	356	175	154	27	— 24,4	< 0,01	35,8
Σ	»	1243	655	264	279	45	— 35,5	< 0,01	37,2

sprechung erheischen. Dass die Spaltung *R* : *rr* in ein paar Fällen merkbar von der erwarteten abweicht ist kaum überraschend, da solche Abweichungen bei genetischen Versuchen mit diesem Allelenpaar oft konstatiert geworden sind. Auch die Verhältnisse *Btb* : *btbbtb* und *Tl* : *tltl* weichen in einigen Kreuzungen ziemlich beträchtlich vom idealen ab. Dass die Spaltung *Pa* : *papa* in Kreuzung XIX so schlechte Übereinstimmung mit dem erwarteten Verhältnis zeigt, beruht auf besondere Umstände. Da ich persönlich gezwungen bin die ganze genetische Klassifikationsarbeit selbst auszuführen, verteilt sich diese trotz aller Anstrengungen über lange Zeit. Unglücklicherweise war Kreuzung XIX die letzte der hierhergehörigen, die 1938 bearbeitet wurde, weshalb die Hülsen zu grossem Teil in der Entwicklung zuweit gekom-

men waren. Dies hat immer zur Folge, dass *papa*-Hülsen verblassen und in gewissem Umfange als *Pa* klassifiziert werden. Die in der Einleitung erwähnte Berechnungsmethode KAPPERTS (FISHERS »test of independence») beabsichtigt indessen für solche Fälle eine Zahlenserie für freie Vererbung mit Rücksicht auf die Mängel in den monohybriden Spaltungen zu schaffen, die mit der gefundenen Zahlenserie verglichen werden kann.

TABELLE 3. *Dihybride Spaltungen in Pa—R, Pa—Tl, Pa—Btb und Tl—Btb.*

Kreuzung		n	Gefundene Anzahl				Differenz für *aabb* (gefunden-erwartet)	P	Crossing-over
Nr.	Typus		*AB*	*A bb*	*aa B*	*aabb*			
XIII	*Pa R* × *pa r*	712	500	31	31	150	+ 104,0	< 0,01	9,0
XVIII	*Pa r* × *pa R*	1075	580	261	233	1	— 56,0	< 0,01	6,9
XV	*Pa ll* × *pa Tl*	158	85	42	30	1	— 7,4	< 0,01	18,1
XVI	»	149	86	38	25	0	— 6,4	»	0 [1]
XVII	»	261	145	56	57	3	— 10,6	»	24,5
Σ	»	568	316	136	112	4	— 24,6	< 0,01	19,7
XIX	*Pa Btb* × *pa btb*	1607	1034	275	188	110	+ 38,6	< 0,01	39,1
XIII	*Pa btb* × *pa Btb*	712	365	166	145	36	— 15,4	< 0,01	33,0
XV	»	158	89	26	36	7	— 2,0	0,5—0,3	43,1
XVI	»	149	78	33	32	6	— 3,9	0,1—0,05	37,3
Σ	»	1019	532	225	213	49	— 21,5	< 0,01	41,5
XV	*Tl Btb* × *ll btb*	158	102	25	23	8	+ 1,5	0,5—0,3	44,2
XVI	»	149	96	28	14	11	+ 4,5	0,05—0,02	38,3
Σ	»	307	198	53	37	19	+ 5,9	0,05—0,02	40,8
XIV	*Tl btb* × *ll Btb*	272	134	75	52	11	— 8,9	< 0,01	36,1

DIHYBRIDE SPALTUNGEN.

Die dihybriden Spaltungen in den Allelenpaaren *R*—*r* und *Btb*—*btb* zeigen, wie aus Tab. 2 hervorgeht, mit wünschenswerter Deutlichkeit, dass *R* und *Btb* gekoppelt sind.

Eine recht erhebliche Variation im Crossingover-Wert zwischen verschiedenen Kreuzungen kann festgestellt werden, aber diese Erscheinung wird hier nicht zur Diskussion aufgegriffen. Der Crossingover-

[1] In Fällen wie diesen gibt FISHERS T_4-Methode offenbar fehlerhafte Resultate.

Prozent ist indessen für die Summe der Kreuzungen vom Typus *R Btb* × *r btb* genau gleich gross wie für die vom reversen Typus.

In Tab. 3 sind die Resultate von Kreuzungen aufgenommen, die gleichzeitig in *Pa R*, *Pa Tl*, *Pa Btb* und *Tl Btb* spalten. Für gewisse dieser Kombinationen ist das Material ziemlich klein, aber dass die betr. dihybride Koppelung zeigen kann als vollkommen sicher betrachtet werden, wenn auch der Wert für *P* in einzelnen Kreuzungen zu hoch ist.

DISKUSSION.

Es steht ausser jeden Zweifel, dass die Gene *Pa* und *Btb*, die von WINGE (l. c.) in seiner Koppelungsgruppe 3 untergebracht worden sind,

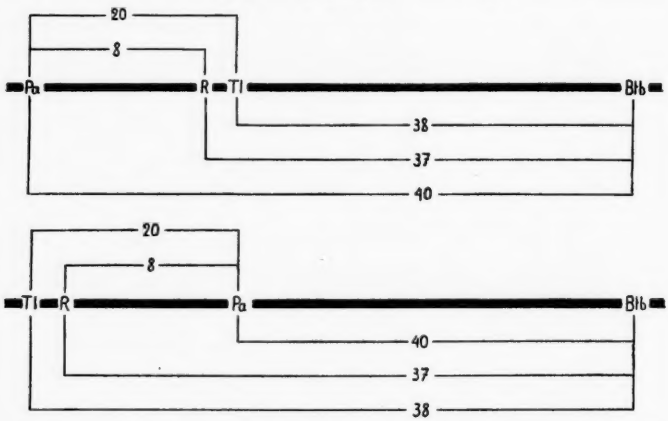

Fig. 2—3. Alternative Genenanordnung in der Koppelungsgruppe *Tl—R—Pa—Btb*.

sowie *R* und *Tl*, allein in Gruppe 7 gestellt, zu einer gemeinsamen Koppelungsgruppe gehören. Ob es als bewiesen erachtet werden kann, dass *N*, das von WINGE zusammen mit *Pa* und *Btb* in Gruppe 3 eingereiht wurde, in dieser untergebracht werden kann, ist vorhin besprochen worden.

Wenn das ganze hier vorgelegte Material berücksichtigt wird, bekommt man eine der Placierungen der Gene, die in Fig. 2 und 3 veranschaulicht sind.

Wie ersichtlich sind die Unterschiede im Crossingover zwischen *Tl* und *Btb*, *R* und *Btb* sowie *Pa* und *Btb* sehr klein, weshalb man gestützt auf das vorliegende Zahlenmaterial nicht entscheiden kann, welcher Anordnung sie in Wirklichkeit entsprechen. In drei Kreuzungen

sind gleichzeitig drei Genpaare studiert worden, nämlich in XIII *Pa*, *R* und *Btb* sowie in XV und XVI *Pa*, *Tl* und *Btb*. In Kreuzung XIII sind folgende Werte für das Crossingover erhalten worden: *R—Pa* 9,0 %, *Pa—Btb* 33,0 % und *R—Btb* 36,8 %. In den Kreuzungen XV und XVI zusammen betragen die gefundenen Werte: *Tl—Pa* 13,7 %, *Tl—Btb* 40,8 % und *Pa—Btb* 41,3 %. Die Resultate in Kreuzung XIII deuten darauf, dass die Gene in der Reihenfolge *Tl*, *R*, *Pa*, *Btb* liegen, aber die Werte für *Pa—Btb* und *R—Btb* sind allzunahe übereinstimmend um bestimmte Schlüsse zuzulassen. Die Kreuzungen XV und XVI geben keine Aufschlüsse von Wert für die Wahl der richtigen Anordnung der Gene. Dass mit Hinblick auf den von anderen Forschern konstatierten sehr kurzen Abstand zwischen *Tl* und *R* der hier gefundene Crossingover-Wert für *R—Pa* im Vergleich mit dem Wert für *Tl—Pa* auffallend klein ist soll jetzt nicht erörtert werden, da in den hier bearbeiteten Kreuzungen keine Zahlen für *Tl—R* vorliegen. Man dürfte sagen können, dass diese Koppelungsgruppe fortwährend viel Forschungsarbeit verlangt.

Die nun beendigten Untersuchungen beweisen nur, dass *Pa—R—Tl—Btb* eine Koppelungsgruppe bilden, und dass *Btb* in grossem Abstand von den übrigen Genen liegt. Laut Untersuchungen von PELLEW (1937) gehört auch *A* zur in Frage stehenden Koppelungsgruppe, trotzdem die Gene *R* und *A* — auf die sich die Untersuchungen bezogen — in gewöhnlichen Fällen etwa 50 % Crossingover zeigen.

DIE GRUPPE Wlo-P-Pl.

LITERATURÜBERSICHT.

Die in der Überschrift genannten Gene sind sämtlich früher veröffentlicht. Das rezessive Allel zu *Wlo* bedingt wachslose Blattoberseite, während alle anderen Teile der Pflanze normal wachsig sind (ERNST NILSSON, 1933 b). Mit *P* wird eines der Gene bezeichnet, die je für sich eine sehr schwache Membran auf der Innenseite der Hülse verursachen, und die zusammen starke Membran und im reifen Zustand glatte Hülse zur Folge haben. WINGE (l. c.) nennt in seiner Übersicht dieses Gen *Pe*, eine Bezeichnung, die ich indessen früher für ein Gen verwendet habe, dessen Abwesenheit die Ursache für petaloide Blüten ist (ERNST NILSSON, 1932). Das dritte Gen, *Pl*, bedingt schwarzes Hilum.

Die Gene *P—Pl* sind laut älteren Untersuchungen gekoppelt (WELLENSIEK, 1927; WINGE, 1936). Noch ein Gen, *Fl*, das in rezessiver Form Blätter und Stipeln ohne graue Panaschierung bedingt, wird als

mit *Pl* gekoppelt betrachtet (H. and O. TEDIN, 1926). Die mit *Fl* ausgeführten Untersuchungen werden hier nicht berücksichtigt, da die Eigenschaft graue Panaschierung in meinem Material Spaltungsverhältnisse gezeigt hat, die ich nicht klarlegen konnte, was bereits in einer früheren Arbeit erwähnt worden ist (ERNST NILSSON, 1933 b). An gleicher Stelle wurde nebenbei erwähnt, dass *Wlo* mit *P* gekoppelt ist, was aus den schon damals ausgeführten Untersuchungen hervorgegangen ist. Im übrigen ist das Verhältnis *Wlo* zu anderen Genen, ausgenommen *Wa* und *Wb* (ERNST NILSSON, 1933 b), die sich unabhängig von *Wlo* herausstellten, nicht untersucht, sowie Cy^2, das starke Koppelung mit *Wlo* zeigte (LAMM, 1937).

In dieser Arbeit werden auch einige andere Gene, die an dihybriden Spaltungen mit den in der Überschrift genannten teilnehmen, behandelt. Die sich auf diese beziehende Literatur wird erst später im Zusammenhang mit den Spaltungen erwähnt werden.

STUDIERTE GENE UND KREUZUNGEN.

Folgende Linien sind zu den Kreuzungen verwendet worden, die in dieser Abhandlung besprochen werden.

L. 15 Niedrige Mai	*Wlo, P, pl*
L. 33 Hamlet	*Wlo, I*
L. 42 Nain mangetout	*Wlo, I, R*
L. 43 Emerald gem	*Wlo, I, R, Le*
L. 49 Luxuria	*Wlo, p, I, R, Le*
L. 54 Acacia	*Wlo, I*
L. 76 Niedrige volltragende	*Wlo, p, pl, I, R*
L. 77 Bohnenerbse	*Wlo, P, Pl, Le*
L. 78 Automobil	*Wlo, P, pl*
L. 85 Chenille-Typus (*s*)	*Wlo, R, Le*
L. 88 » »	*Wlo, R, Le*
L. 89 *wlo*-Linie aus English Wonder	*wlo, P, pl, i, r, le*
L. 91 Blue Peter	*Wlo, R*
L. 92 Meteor	*Wlo, R*
L. 93 De Grace	*Wlo, p, pl, I, R, le*
L. 94 Rekord	*Wlo, R*
L. 97 Rogues-Typus aus Viking	*Wlo, I, R*
— *le Pl*-Linie	*Wlo, Pl*
— *le wlo*-Linie	*wlo, P*
— *wlo p pl*-Linie	*wlo, p, pl*
— *Wlo p Pl-Linie*	*Wlo, p, Pl*

Die bearbeiteten Kreuzungen sind hier unten zusammengestellt, wobei reziproke Kreuzungen zur gleichen Nummer gerechnet worden sind.

Kreuzung			
Kreuzung	I. L. 49 × L. 89	*Wlo p R I Le*	× *wlo P r i le*
»	II. L. 76 × »	*Wlo p R I*	× *wlo P r i*
»	III. L. 93 × »	*Wlo p R I*	× *wlo P r i*
»	IV. L. 49 × *le wlo*	*Wlo p*	× *wlo P*
»	V. L. 77 × L. 89	*Wlo Pl Le*	× *wlo pl le*
»	VI. *le Pl* × »	*Wlo Pl*	× *wlo pl*
»	VII. L. 77 × L. 93	*P Pl Le*	× *p pl le*
»	VIII. *le Pl* × L. 76	*P Pl*	× *p pl*
»	IX. L. 43 × L. 89	*Wlo R I Le*	× *wlo r i le*
»	X. L. 88 × »	*Wlo R I Le*	× *wlo r i le*
»	XI. L. 42 × »	*Wlo R I*	× *wlo r i*
»	XII. L. 85 × »	*Wlo R Le*	× *wlo r le*
»	XIII. L. 97 × »	*Wlo R I*	× *wlo r i*
»	XIV. L. 91 × »	*Wlo R*	× *wlo r*
»	XV. L. 92 × »	*Wlo R*	× *wlo r*
»	XVI. L. 94 × »	*Wlo R*	× *wlo r*
»	XVII. L. 33 × »	*Wlo I*	× *wlo i*
»	XVIII. L. 54 × »	*Wlo I*	× *wlo i*
»	XIX. L. 77 × *wlo p pl*	*Wlo P Pl*	× *wlo p pl*
»	XX. L. 78 × »	*Wlo P*	× *wlo p*
»	XXI. L. 77 × L. 49	*P Pl*	× *p pl*
»	XXII. L. 15 × *Wlo p Pl*	*P pl*	× *p Pl*

Ein Teil dieser Kreuzungen ist identisch mit gewissen früher veröffentlichten (ERNST NILSSON, 1933 b), haben aber hier andere Nummern. Die Linien haben indessen sowohl in dieser wie in der früheren Arbeit die gleiche Bezeichnung, weshalb eine Identifizierung keine Schwierigkeiten verursacht.

DIE SPALTUNGEN.

In Tab. 4 sind die wichtigsten Daten hinsichtlich der studierten Kreuzungen zusammengestellt. Da einige Kreuzungen in F_2 eine zu geringe Individuenanzahl hatten um eine separate Behandlung zu motivieren, sind alle Kreuzungen vom gleichen Typus summarisch bearbeitet.

Einige der monohybriden Spaltungen weichen beträchtlich vom

idealen Verhältnis ab. Besonders gilt dies für die Spaltung *Le* : *le le*, für die der Quotient D:m_k 3 oder noch mehr beträgt. Der Ausfall an Rezessiven dürfte in diesen Fällen sicherlich auf geringerer Konkurrenzkraft des niedrigen Typus in einem gemischten Bestand beruhen. Eine zu kleine Anzahl *le*-Pflanzen im Verhältnis zu der erwarteten ist übrigens eine häufige Erscheinung, die von mehreren Forschern konstatiert worden ist.

TABELLE 4. *Dihybride Spaltungen in Wlo—P, Wlo—Pl, P—Pl und einige andere Gene.*

Kreuzung		n	Gefundene Anzahl				Differenz für *aabb* (gefunden-erwartet)	P	Crossing-over	A		B	
Nr.	Typus		*AB*	*Abb*	*aaB*	*aabb*				Rezessive pro 4	D:m_k	Rezessive pro 4	D:m_k
XIX—XX	*Wlo P* × *wlo p*	1977	1421	110	67	379	+ 268,7	< 0,01	19,6	0,90	2,7	0,99	0,3
I—IV	*Wlo p* × *wlo P*	1263	639	340	283	1	— 75,7	»	5,8	0,90	2,0	1,08	1,6
V—VI, XIX	*Wlo Pl* × *wlo pl*	1451	915	202	179	155	+ 72,8	»	32,1	0,92	1,8	0,98	0,4
VII—VIII, XIX, XXI	*P Pl* × *p pl*	3057	1929	416	383	329	+ 155,5	»	31,9	0,93	2,2	0,97	1,0
XXII	*P pl* × *p Pl*	237	119	54	60	4	— 11,7	»	25,0	1,08	0,7	0,98	0,2
I, V, IX, X, XII	*Wlo Le* × *wlo le*	1935	861	215	249	70	+ 4,8	0,5—0,3	—	0,91	1,9	0,82	3,9
VII	*P Le* × *p le*	263	162	37	56	8	— 3,0	0,3—0,2	—	0,97	0,3	0,68	3,0
I	*P le* × *p Le*	371	196	67	84	24	— 5,2	0,2—0,1	—	1,16	1,8	0,98	0,2
VII	*Pl Le* × *pl le*	263	165	39	53	6	— 4,1	0,2—0,1	—	0,90	0,9	0,68	3,0
I—III, IX, XVI	*Wlo R* × *wlo r*	3441	1978	615	642	206	+ 3,7	0,8—0,7	—	0,99	0,3	0,95	1,7
I—III, IX—XI, XIII, XVII—XVIII	*Wlo I* × *wlo i*	2673	1480	524	507	162	— 9,7	0,5—0,3	—	1,00	0,00	1,03	0,9

Dass *Wlo* starke Koppelung mit *P* und ziemlich starke mit *Pl* zeigt ist offenbar. Auch *P* und *Pl* zeigen ziemlich starke Koppelung, was schon früher von WELLENSIEK (1927) und von WINGE (l. c.) festgestellt worden ist. Die Gene *Wlo*, *P* und *Pl* gehören demnach mit voller Sicherheit zur gleichen Koppelungsgruppe.

Laut vorliegender Untersuchung zeigt *Wlo—P* durchschnittlich 12,7 %, *P—Pl* 28,5 % und *Wlo—Pl* 32,1 % Crossingover, weshalb die gegenseitige Lage der Gene die in Fig. 4 wiedergegebene sein dürfte. Ausgeschlossen erscheint jedoch nicht, dass die Anordnung auch *P—Wlo—Pl* sein könnte.

Berechnet laut HALDANES Formel (zit. laut SANSOME and PHILP,

1932, S. 113) sollte der Abstand zwischen *Wlo* und *Pl* mit den gefundenen Abständen zwischen *Wlo* und *P* sowie *P* und *Pl* 33,4 % betragen, wenn doppeltes Crossingover in der erwarteten Ausdehnung vorkommt, jedoch 41,2 % wenn kein doppeltes Crossingover auftritt. Wie ersichtlich stimmt der erstgenannte Wert ziemlich gut mit dem gefundenen überein.

WELLENSIEK (1927) fand in einer Kreuzung vom Typus $AB \times ab$, berechnet laut FISHERS T_4-Methode, einen Crossingover-Procent für

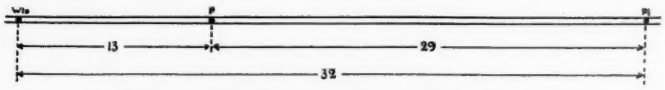

Fig. 4. Die Koppelungsgruppe *Wlo—P—Pl*.

P—Pl von 32,3 und WINGE (l. c.) in der Kreuzung vom Typus $AB \times ab$ 32,9 % und für $Ab \times aB$ 36,2 %. Meine Resultate, die in Kreuzungen $AB \times ab$ erhalten worden sind, stimmen mit obenstehenden vom gleichen Typus ziemlich gut überein, wogegen WINGES Kreuzung vom reversen Typus schwachere Koppelung zeigt als meine entsprechende Kreuzung.

Von den Kreuzungen I und II ist auch eine ziemlich grosse Anzahl Familien in F_3 untersucht worden. Die Nachkommen von *WloP*-Individuen in F_2 haben sich in F_3 in folgender Weise verteilt.

	Spalt. *Wlo* Spalt. *P*	Spalt. *Wlo* Konst. *P*	Konst. *Wlo* Spalt. *P*	Konst. *Wlo* Konst. *P*
Theoretisches Verhältnis:	$2n^2 + 2$	2n	2n	1
Gefundene Anzahl Familien: ..	129	5	8	2
Erwartete Anzahl Familien: ..	126,7	8,5	8,5	0,3

$\chi^2 = 11{,}153$, $P = 0{,}02 - 0{,}01$

Der für das Crossingover gefundene Wert, 6,3 %, entspricht einem Gametenverhältnis von ungefähr 1 : 14,9. Man sieht indessen, dass die gefundenen Zahlen den erwarteten schlecht entsprechen. Untersucht man die hier nicht aufgenommenen Detailberechnungen, findet man, dass dies hauptsächlich auf einen Überschuss in den Klassen »Konst. *Wlo*—Konst. *P*» beruht. Die zwei Familien, die hierher gerechnet worden sind, bestanden indessen nur aus 2 bzw. 3 Individuen — die niedrigsten Individuenzahlen sämtlicher Familien. Man kann also nicht mit der geringsten Spur von Sicherheit behaupten, dass diese zwei Familien wirklich in diese Klasse gehören. Man dürfte auch mit vollem Recht die Grenze für die aufgenommenen Familien so hoch ziehen,

dass Familien mit so geringer Individuenanzahl ausgeschlossen werden. Dann bekommt man das folgende Resultat, das der genetischen Wirklichkeit besser und überdies den erwarteten Verhältnissen entsprechen dürfte.

	Spalt. *Wlo* Spalt. *P*	Spalt. *Wlo* Konst. *P*	Konst. *Wlo* Spalt. *P*	Konst. *Wlo* Konst. *P*
Theoretisches Verhältnis:	$2n^2 + 2$	2n	2n	1
Gefundene Anzahl Familien: ..	129	5	8	0
Erwartete Anzahl Familien: ..	125,0	8,4	8,4	0,3

$\chi^2 = 1{,}658$, $P = 0{,}7 - 0{,}5$

Im übrigen ist zu bemerken, dass der Unterschied im Crossingover zwischen den Kreuzungen *Wlo P* × *wlo p* und *Wlo p* × *wlo P* auffallend gross ist. Die Individuenanzahl ist in beiden Fällen gross, weshalb der Unterschied nicht ohne Bedeutung sein dürfte. Hierzu kommt, dass bei Bearbeitung der verschiedenen Kreuzungen je für sich die Übereinstimmung in den betr. Koppelungs- bzw. Repulsionsgruppen gut ist. Kreuzung XIX gibt 19,8 und XX 19,3 % Crossingover, während die Kreuzungen vom Repulsionstypus folgende Resultate geben: II 9,7 % sowie I, III und IV je für sich 0 % (keine doppeltrezessiven Individuen), d. h. sehr starke Koppelung zwischen *Wlo* und *p*. Der in diesen Fällen vorhandene grosse Unterschied zwischen den reversen Kreuzungen soll indessen einstweilen nicht zum Gegenstand einer Diskussion gemacht werden. Es sei nur hinzugefügt, dass die Kreuzungen I—IV hauptsächlich 1932 und die Kr. XIX—XX 1938 bearbeitet worden sind, ohne deshalb zu behaupten, dass die Witterung der verschiedenen Jahre die Ursache der Unterschiede gewesen ist, wenn dies auch der Fall *sein könnte*.

In Tab. 4 sind auch die Resultate für die Untersuchung von *Wlo* in dihybriden Spaltungen zusammen mit *Le*, *R* und *I* aufgenommen. In keinem Fall liegt hier eine Andeutung für Koppelung vor.

Da WINGE auch *Le* in die gleiche Koppelungsgruppe wie *P* und *Pl* einreiht, zu der *Wlo* gehört, sind auch die vorliegenden Zahlen für *P—Le* und *Pl—Le* aufgenommen worden. Beide diese Kombinationen zeigen hier solche Resultate, dass man eine unabhängige Vererbung oder Koppelung mit sehr hohem Crossingover annehmen muss.

Hinsichtlich *Pl—Le* haben sowohl KAPPERT (1925), WELLENSIEK (1925 b), WINGE (l. c.) und LAMPRECHT (1933) freie Vererbung festgestellt, während die Ansichten in bezug auf *P—Le* geteilt sind. RASMUSSON (l. c.) hat in verschiedenen Kreuzungen variierendes

Crossingover gefunden, während WINGE (l. c.) und WELLENSIEK (1925 b) keine Andeutung zu Koppelung konstatieren konnten.

Eine dihybride Spaltung, die in diesem Zusammenhang auch von Interesse ist, bezieht sich auf die Gene *Wlo* und *V*, die in Kreuzung XI, aber erst in F_3 und an 378 Individuen studiert worden ist. Es hat keine Andeutung von Koppelung zwischen *Wlo* und *V* beobachtet werden können ($P = 0{,}7 - 0{,}5$).

Gewisse Gründe sprechen dafür, dass die bekannte Koppelungsgruppe *V—Le* und die hier behandelte, *Wlo—P—Pl*, zu einer gemeinsamen Gruppe gehören. RASMUSSON (l. c.) hat hier grundlegende Untersuchungen ausgeführt, und ein Zusammenhang ist speziell via das Gen *Bta* konstatiert worden. Dass die früher untersuchten Verhältnisse zwischen *Wlo* und *Le* sowie *Wlo* und *V* in meinem Material keine Andeutung von Koppelung gegeben haben braucht nicht zu überraschen, da *Wlo* sehr nahe *P* liegt, und der Abstand zwischen *P* und *Le* bzw. *V*, insofern *P* zur gleichen Gruppe wie diese gehört, sehr gross ist. Sicher ist indessen, dass WINGES Gruppe 2 noch zum Gegenstand eingehender Erforschung zu machen ist, bevor die Kenntnis derselben als befriedigend zu betrachten ist.

ZUSAMMENFASSUNG.

1. Die Arbeit behandelt das Ergebnis einer Serie von Untersuchungen in bezug auf die Gene *Pa, R, Tl, Btb* und *Wlo, P, Pl*. Hinsichtlich *Wlo* ist früher sein Zusammenhang mit *Wa, Wb* und Cy^2 bekannt.

2. Koppelung ist nachgewiesen worden zwischen *Pa—R* mit 7—9 %, zwischen *Pa—Tl* mit 20 %, zwischen *R—Btb* mit 37 % und zwischen *Tl—Btb* mit 36—41 % Crossingover. Hieraus folgt, dass *R—Tl* und *Pa—Btb* zur gleichen Koppelungsgruppe gehören.

3. Das vom Verf. früher beschriebene Gen *Wlo* zeigte sich im vorliegenden Material zu 6 % bzw. 20 % Crossingover mit *P* und zu 32 % Crossingover mit *Pl* verbunden. *P—Pl* waren mit 25 bzw. 32 % Crossingover gekoppelt, eine Erscheinung, die ziemlich gut mit Resultaten älterer Untersuchungen übereinstimmt. *Wlo—P—Pl* gehören also zu einer Koppelungsgruppe.

4. Die bisher nicht untersuchten dihybriden Kombinationen *Wlo—R, Wlo—I, Wlo—Le* und *Wlo—V* haben keine Andeutung für Koppelung gezeigt, was auch für die früher bekannten Kombinationen *P—Le* und *Pl—Le* gilt.

Hammenhög im August 1938.

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SELECTIVE FERTILIZATION IN POULTRY

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THE question as to whether fertilization in poultry can be selective has been treated by DUNN (1927), whose experiments to a certain degree seemed to indicate the occurrence of such selectivity. Related to this question is the problem of competition between different spermatozoa, for instance as occurring at insemination when different cocks have been used alternately. Several investigators (CREW, 1926; WARREN and KILPATRICK, 1929; KRALLINGER, 1930) have shown that as a rule the latest insemination is the fertilizing one. The investigators mentioned worked with natural insemination. If artificial insemination is used the possibilities of controlling the details of the experiment are increased. For this reason we have taken up the questions indicated with the aid of this technique. (A detailed report on our experiments with artificial insemination will be given in another connection.)

The experiments were carried out with two parallel groups, I and II. Each group consisted of 5 Rhode Island Red hens and 2 cocks, one Rhode Island Red, the other White Leghorn. As the White Leghorns have dominant white, the colour of the offspring will show which of the two cocks has yielded the fertilizing sperm. The plan was that every individual hen was to be inseminated on the afternoon of every day when she had laid an egg. However, a few exceptions from this rule were made, so that in some cases a hen was inseminated without having laid an egg on the day of insemination. Also it occurred that some of the eggs were broken and therefore not put into the incubator. The experimental details will be found in Table 1. All fertilized eggs which were not hatched were opened and, if possible, identified in regard to paternity.

In group I the hens were inseminated alternately with sperm of Rhode Island and sperm of White Leghorn from the beginning of the investigation on 23rd February 1938 to the end of March. During April insemination was made each time with mixed sperm, taken in equal parts from each cock (see Table 1 for details). The mixing was carried out by sucking up and down with a pipette. The whole experiment comprised 210 eggs in Group I, 3 of which were infertile, while 17 had died so early that they could not be identified.

TABLE 1. *Detailed results of the experiment. Corresponding to eac*hen
*R = red chick, W = white chick, d = embryo dead too early fo*lden
*I = semen from white Leghorn cock, i = semen from red Rhode Islan*cock
*which, according to the insemination*were

Group	Hen	February				March											10
		23	24	25	26	27	28	1	2	3	4	5	6	7	8	9	
I	H 287	i		R I		W i	W I		W i	W I	R i		R I	d i	W I		W i
	H 860		I	n i		n I	R i	W I		W i	R§ I	R i		R I	W§ i		R I
	H 1182		I	n i		R I	R i		R I		W i		d I	d i		d I	R i
	H 1195																
	H 1208	i	n I		R§ i	R§ I		W i	W I		W i		W§ I	R i		R I	R i
II	H 1166		i	n I		R i		R I		d i	W I	R i	R I		R i	R I	R i
	H 1176	i		R I				n i	R I		R i		R I		i	W I	
	H 1177	i		n I	R i		R I	R i		R I		W i	d I		R i	R I	
	H 1194		i		n I	R i	d I		R i	R I	R i		W I	R i	R I		R i
	H 1215																

Group	Hen	March				April											10
		26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	
I	H 287	W	R§ I	R§ i		R I	d i	W m	d m		d m	W m	W m		W m	R m	
	H 860	W I		R§ i	W I	R i		W§ m	W§ m		W m	W m	R m	R m		W m	R m
	H 1182	R§ I		R§ i	W I	R i	W I		n m	R§ m	R m	R m	R m	R m		R m	R m
	H 1195	R§ i	W I		R§ i	W I		R§ m	W m	W m		R m	R m	R m		R m	W m
	H 1208	R i	W I		W i	R§ I	R i		R m	R m	W m	R m		W m	R m	R m	R m

ng to eac hen there are two lines. The upper line shows the fate of the eggs:
early fo dentification, n=infertile egg. The lower line shows the inseminations:
ode Islan ock, m = mixed semen. The sign § in group I indicates those chicks
minations ere expected to be of the other colour.

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
W I		W i	W I		R§ i	W I	R i		R I	R i	W I		W i	W I	R i	W I	
W§ i		R I	R i	W I		i	W I	R i		R I	R i	W I		R§ i	W I		R§ i
	d I	R i	W I	R i		W§ I	W§ i	R§ I		R§ i	R§ I	R i	R§ I		W i	R§ I	R i
						i		R I	i		R I	d i		d I	R i	W I	
	R I	R i	W I		R§ i	R§ I	R i		R I	R i	W I		R§ i	W I		W i	W I
R i	R I	R i	R I			R i	R I	R i		R I	R i		R I	R i	R I		R I
	W I		R i	R I		R i	R I	R i		R I	R i		R I	n i	R I		R i
R i	R I		R i	R I		R i	d I	R i		R I		R i	R I	R i		d I	R i
R I		R i	R I	R i	R I		R i	R I		W i	W I	R i	R I	R i		R I	R i
						I	n i		R I		W i		R I	R i		R I	R i

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
W m	R m		R	R m	W m		d m								m		m
	W m	R m	R m		R m	R m	R m		d m	R m	R m		W m	R m		R m	
	R m	R m			R m	R m	R m	R m		R m	R m	R m	R m	R m	R m		R m
	R m	W m	R m		W m	d m	d m		d m	R m	R m	d m		d m	W m		d m
R m	R m	R m	R m		R m	R m	W m	m		R m	W m	R m	R m		R m	W m	R m

TABLE 1. (Continued.)

Group	Hen	March						April								
		26	27	28	29	30	31	1	2	3	4	5	6	7	8	9
II	H 1166	d I	R i	R I	R i			R m	R m		R m	R m	R m		R m	R m
	H 1176		R I			R i	R I	R m		R m	R m	R m	R m		R m	R m
	H 1177		R I	R i												
	H 1194	R I	R i	R I	R i	R I										
	H 1215		R I	R i	R I		R i	R m		R m	R m		R m	R m	R m	

Group	Hen	April				May										
		26	27	28	29	30	1	2	3	4	5	6	7	8	9	10
I	H 287	n m		W m												
	H 860	R m		R m	R m	W										
	H 1182	m	R m	R m												
	H 1195	R m	R m		W m	W										
	H 1208		W m	W m	W											
II	H 1166	d I	n I	n I	n I	n I		I	n I	n I	n I	n I	n I		n I	
	H 1176	n I		n I	n I		n I	n I	n I		n I	n I	n I		n I	
	H 1177	n I	n I	W I		W I	n I	W I	W I		W I	n I		n I	W I	n I
	H 1194		W I	n I	n I	n I	W I	I	W I	d I		W I	n			
	H 1215	R I		W I	W I		W I	n I		n I	n I	n I		n I	W I	n I

7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	R m	R m	R m	R m	n m	R m	R m	R		R I		I	R I	R I	I		R I	R I
	R m	R m		R m	R m	d m		R I	R I	R I	R I	R I	R I	R I	R I		d I	n I
								I		W I	n I	n I		W I	n I	n I		W I
									I	n I		W I	n I	n I	n I	n I	n I	W
m	R m		R m	R m		R m	R m	R I		R I	R I		R I	R I	R I		I	R I

9	10	11	12	13	14	15
n I			n I	n I		n
n I		n	n I	n I		n
W I	n I					
W I	n I	n I	W			

For a closer analysis of the question as to whether only the latest insemination made is to be regarded as fertilizing, Group I may be studied for the first period (when insemination was carried out alternately from the two cocks), by calculating the number of white chickens that, according to this assumption, are expected to be white and the number expected to be red; and a corresponding calculation made for the red chickens. However, it must be borne in mind that if an insemination is made on a certain day, and if an egg is laid on the next day, it is only in exceptional cases that the egg will be fertilized by sperm from this insemination. In all our (not yet published) experiments we have 94 eggs laid 1 day after insemination when no earlier insemination had been made (or made so long ago that it need not be taken into account). Of these only 3 were fertilized. Our results show that the highest frequency of fertilization is to be found two days after insemination. In Table 1 the sign § has been placed after each egg where the chicken hatched was not of the colour corresponding to the latest insemination, *not* taking into account the insemination made only *one* day before the egg was laid. The error thus introduced in any case must be small. If the different kinds of fertilization for each hen are added up, the result shown in Table 2 will be obtained. As mentioned above, insemination with mixed sperm was begun on 1st April, but here also regard has been paid to the fact that only in exceptional cases does fertilization occur earlier than two days after insemination, and for each hen, consequently, the eggs laid

TABLE 2. *Results from group I, during the time when insemination was performed alternately with sperm from the white and the red cock.*

Hen No.	White chicks which, according to insemination, are expected to be		Total white chicks	Red chicks which, according to insemination, are expected to be		Total red chicks	Total identified chicks	Embryos dead too early for identification	Total fertile eggs	Infertile eggs
	white	red		white	red					
H 287 ...	14	0	14	3	8	11	25	3	28	0
H 860 ...	8	3	11	4	9	13	24	0	24	1
H 1182 ...	5	2	7	8	8	16	23	3	26	1
H 1195 ...	4	0	4	3	3	6	10	2	12	0
H 1208 ...	10	1	11	6	10	16	27	0	27	0
Total	41	6	47	24	38	62	109	8	117	2

have been counted in Table 2 up to the corresponding correct date in April. In Table 1 this date is indicated by a vertical line. For the same reason, in the construction of Table 2 and subsequent tables the infertile eggs laid on 24th February by hen H 1208, on 25th February by H 860, H 1182, H 1166, on 15th March by H 1215, and on 17th April by H 1194, have not been included.

TABLE 3. *Results from group I, during the time when insemination was performed with sperm mixed from the two cocks.*

Hen No.	White chicks	Red chicks	Total identified chicks	Embryos dead too early for identification	Total fertile eggs	Infertile eggs
H 287	5	3	8	2	10	1
H 860	5	14	19	1	20	0
H 1182	0	19	19	0	19	0
H 1195	6	9	15	6	21	0
H 1208	8	12	20	0	20	0
Total	24	57	81	9	90	1

It will be seen from Table 2 that out of 47 white chickens 6 should have been red according to the date of insemination. This indicates that as a rule the latest insemination is responsible for fertilization, but

TABLE 4. *Test of homogeneity to Table 3.*

Homogeneity based on all identified chicks			Homogeneity when non-identified embryos are added to the red chicks		
χ^2	Degrees of freedom	P	χ^2	Degrees of freedom	P
14,0509	4	0,0072	11,5787	4	0,0212

that a certain number of exceptions occur. Of the 62 red chickens no less than 24 should have been white; here the discrepancies are more common. This greater relative fertilizing capacity of the red spermatozoa may of course have been due to a certain percentage of defective spermatozoa in the sperm of the white cock. The sperm from the two cocks, however, was from time to time controlled under the microscope. No countings or other detailed examinations were made, but the sperm from both cocks gave the impression of full vitality.

The conditions in Group I during the period when the sperm from the two cocks was mixed, are shown in Table 3. If there was no predominance in fertilizing capacity of the spermatozoa of one kind, one would expect the same number of white and red chickens. But out of 81 chickens 24 are white and 57 red, which cannot be 1 : 1. Even if all the 9 unidentified chickens are counted as white, *i. e.* if the proportion is taken to be 33 : 57, this cannot be regarded as 1 : 1, as the probability of this is only $P = 0{,}0114$. Table 3 further demonstrates the very interesting fact that fertilization is not only selective in favour of the red cock, but also that selectivity obtains when the hens are compared among themselves. In Table 4 the homogeneity of the proportion of the red and white chickens has been tested; it will be seen that a marked *heterogeneity* obtains. As a matter of fact one hen (H 1182) has had only red chickens from insemination with mixed sperm. All eggs from this hen were identified. It might be thought that if the unidentified chickens from the other hens had all been red, this heterogeneity would disappear. In Table 4 an analysis has been made under this assumption also, but the heterogeneity still remains marked.

The hens used were Rhode Island Red, and the cock which predominated in the competition was of the same race. Whether the difference is to be ascribed only to individual dissimilarities between the two cocks, independent of racial characters, or whether the reason is that the sperm from the Rhode Island cock is »race-specific» in relation to the hens, cannot yet be said. On the whole, the causes underlying our results cannot be decided at present, but it apppears as if the problem is of great interest.

Originally our intention was to carry out exactly the same tests with the animals in Group II as in Group I. When the hatchings from the inseminations of the first period had been going on for some time, however, it became evident that in this group the fertilizations were mainly due to the Rhode Island cock. Consequently it was decided to shorten the mixed sperm period — originally intended to last the whole of April, as for Group I — to only two weeks, and then to inseminate only with sperm from the White Leghorn cock. On microscopic examination the sperm from this cock was frequently found to be of poor quality, containing a greater or smaller quantity of immobile spermatozoa. However, no detailed microscopic study of the sperm was made in this case either. Also, on several occasions the amount of sperm from this cock was too small; the sperm was then diluted to

the proper quantity. (As regards fluids for dilution we refer to our future report on the experiments with artificial insemination.) When the insemination was carried out with sperm mixed from both the cocks, the dilution — when necessary — was made after equal quantities of sperm had been mixed.

TABLE 5. *Results from group II, during the time when insemination was performed alternately with sperm from the white and the red cock.*

Hen No.	White chicks	Red chicks	Total identified chicks	Embryos dead too early for identification	Total fertile eggs	Infertile eggs
H 1166	1	22	23	2	25	0
H 1176	1	18	19	0	19	2
H 1177	1	17	18	3	21	1
H 1194	3	22	25	1	26	1
H 1215	1	10	12	0	11	0
Total	7	89	96	6	102	4

TABLE 6. *Results from group II, during the time when insemination was performed with sperm mixed from the two cocks.*

Hen No.	White chicks	Red chicks	Total identified chicks	Embryos dead too early for identification	Total fertile eggs	Infertile eggs
H 1166	0	11	11	0	11	1
H 1176	0	10	10	1	11	0
H 1215	0	10	10	0	10	0
Total	0	31	31	1	32	1

Table 5 shows the results from the period when sperm was inseminated alternately from the two cocks (for the same reasons as in the case of Group I this period has been deferred to the date in April marked by a vertical line in Table 1). That the sperm from the white cock — no doubt on account of its poor quality — was definitely outclassed by that from the red cock, is obvious. And Table 6 shows that during the period (likewise marked by a vertical line in Table 1) when mixed sperm was inseminated, the sperm from the white cock was entirely suppressed. At the beginning of this period two of the hens

(H 1177 and H 1194) became broody. When they started laying again — and consequently had only sperm from the white cock — many of the eggs were infertile (Tables 1 and 7). From the others white chickens were of course hatched. The three hens that did not become broody went on for some time, as was to be expected, to produce red chickens.

TABLE 7. *Results from group II, during the time when insemination was performed with sperm from the white cock only.*

Hen No.	White chicks	Red chicks	Total identified chicks	Embryos dead too early for identification	Total fertile eggs	Infertile eggs
H 1166	0	4	4	1	5	13
H 1176	0	6	6	1	7	15
H 1177	9	0	9	0	9	10
H 1194	6	0	6	1	7	9
H 1215	5	7	12	0	12	7
Total	20	17	37	3	40	54

TABLE 8. *White chicks and infertile eggs from Table 7.*

Hen No.	White chicks	Infertile eggs	Total
H 1166	0	13	13
H 1176	0	15	15
H 1177	9	10	19
H 1194	6	9	15
H 1215	5	7	12
Total	20	54	74

TABLE 9. *Test of homogeneity to Table 8.*

χ^2	Degrees of freedom	P
16,9406	4	0,0020

After that there followed infertile eggs or white chickens. As all white chickens or infertile eggs respectively, as regards time, came *after* the red chickens, the white and the infertile eggs may be collocated separately from Table 7, which has been done in Table 8. Also a homogeneity analysis can be carried out for this table; it will be found in Table 9. It has not been possible to take into account the embryos which died too early for identification, but this cannot affect the conclusions. It will be seen that the heterogeneity is very marked, the deficient sperm of the Leghorn cock being capable of fertilizing some

eggs in certain hens but not in others, although according to Tables 5 and 6 these latter are fully capable of fertilization. It can thus be concluded that, as assumed by DUNN, a definite selectivity is evident at fertilization in poultry.

SUMMARY.

1. With the aid of the artificial insemination technique experiments have been carried out on fertilization in hens. Two parallel groups were employed, each consisting of 5 Rhode Island Red hens, 1 Rhode Island Red cock, and 1 White Leghorn cock.

2. With the first group the following tests were carried out:

During a first period: inseminations alternately from the red and from the white cock. In accordance with the results of other investigators it was found that as a rule the latest insemination was responsible for fertilization. Deviations from this rule, however, were more common in the case of the sperm of the red cock than in that of the white one.

During a second period: inseminations with sperm mixed in equal parts from both the cocks. The result was not only a greater number of fertilizations by the red cock than by the white, but besides, this surplus of fertilizations by red sperm differed significantly between different hens. From one of the hens only red chickens were obtained.

Simple microscopic control of the sperm gave the impression that it was equally vital in both cocks.

3. In the second group the tests were:

During a first period: inseminations alternately from the red and the white cock. The result was a very large surplus of fertilizations by the red cock.

During a second period: inseminations with sperm mixed in equal parts from both cocks. Only red chickens were hatched.

During a third period: inseminations with sperm from the white cock alone. When the remaining red sperm had ceased to be effective, some of the hens produced only infertile eggs, others infertile eggs and white chickens. During the first two periods all the hens had proved themselves to be fully capable of fertilization, but during the third period the fertility differed significantly for the different hens.

Simple microscopic control of the sperm gave the impression that it was fully vital in the red cock, but of low quality and containing large quantities of immobile spermatozoa in the white cock.

4. The conclusions that may be drawn are:

a. Competition between the spermatozoa from different cocks may give rise to selective fertilization even in cases where, according to simple microscopic controls, the sperm seems to possess full and equal vitality. It cannot at present be decided whether this selectivity is to be regarded as a difference between individual cocks, independently of race, or whether racial similarity plays any part.

b. This selectivity does not depend only on the different kinds of sperm, as hens may react differently towards different sperm.

c. Hens which have proved fully capable of fertilization when inseminated with vital sperm, may exhibit different degrees of fertility when inseminated with sperm of low quality.

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CYTOLOGICAL STUDIES IN A HAPLOID PLANT OF TRITICUM VULGARE

BY *N. KRISHNASWAMY*
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THE first haploid plant in wheat was reported by GAINES and AASE (1926) in an F_1 of an intergeneric cross *Triticum compactum Humboldii* × *Aegilops cylindrica*. Since then the occurrence has been recorded in all the other wheat groups also. GAINES and AASE observed 21 chromosomes in the somatic cells and usually 21 univalents with the occasional occurrence of 1—2 bivalents at meiosis.

In the haploid *Tr. monococcum*, KIHARA and KATAYAMA (1933) reported 7 univalents and the occurrence of 1 bivalent at the first metaphase, in up to two per cent of the pollen mother cells. CHIZAKI (1933) again recorded a haploid in the same species but did not report on the meiosis. KATAYAMA (1934, 1935), also in *Tr. monococcum*, reported the spontaneous occurrence of haploid plants in the field up to 0,48 % and obtained up to 17,58 % haploids by using X-rayed pollen on normal pistils. He also observed the occurrence of bivalents but paid particular attention to the movement of the univalents after the first meiotic division.

YEFEIKIN and VASILYEV (1936), by employing X-rayed pollen, also obtained haploids in *Tr. dicoccum* and *Tr. persicum* but the heterotypic division was not studied.

In the F_1 of the interspecific cross *Tr. durum* × *Tr. monococcum*, VASILYEV (1936) obtained a haploid plant of *Tr. durum*. The meiotic behaviour was not reported. In the same species KIHARA (1936) found in 7 twin-seedlings one pair to be diplo-haploid. He observed 3 as the highest number of bivalents per pollen mother cell.

NAKAJIMA (1935) reported the occurrence of a haploid plant along with a hybrid in the intergeneric cross *Triticum turgidum* × *Secale cereale*. He observed 14 chromosomes in the root-tips but could not study the meiosis.

In individual plant progenies of *Tr. spelta* var. *albospicatum*, SIMONET and FLECKINGER (1937) discovered two haploid plants. The somatic cells gave a count of 21 chromosomes. The authors record the presence of one chromosome with a large subterminal constriction,

»mais ici, seul, un élément présente une profonde constriction subterminale à l'une de ses deux branches» (p. 26).

The first haploid plant in *Tr. vulgare* was observed by YAMASAKI (1934) as a mutant, and he counted 21 chromosomes in the somatic cells. In the same year NAMIKAWA and KAWAKAMI reported the occurrence of a haploid among twin-seedlings. YAMASAKI (1936) and YAMAMOTO (1936) reported on the behaviour of the meiosis of the haploid *Tr. vulgare*.

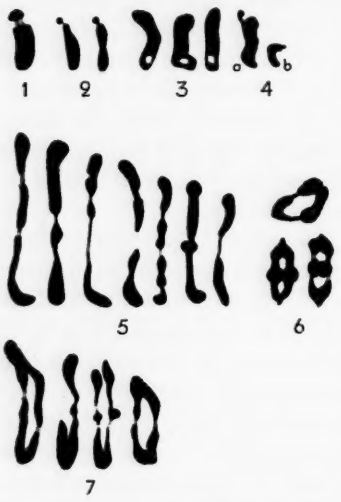

Fig. 1. Univalent with a thick satellite-like body. (× ca. 2170). — Fig. 2. Univalents with a satellite. (× ca. 2170). — Fig. 3. Univalents with an achromatic portion. (× ca. 2170). — Fig. 4 *a*. Univalent with the satellite attached subterminally. (× ca. 2170). — Fig. 4 *b*. Univalent with a pointed end. (× 1500). — Fig. 5. Different types of bivalents. (× ca. 2170). — Fig. 6. Ring-bivalents. (× ca. 2170). — Fig. 7. Trivalents. (× ca. 2170).

The present case is the first instance of a haploid *Tr. vulgare* in Europe. The origin of this plant is from a twin-seedling obtained from the Seed-control station at Stockholm and raised in Svalöf, in September 1937. The other member of the pair died. The root-tip cells gave a count of 21 chromosomes. The meiotic stages were fixed in July 1938 in modified Karpechenko's fluid. Paraffin sections, 20 μ thick, were cut and stained in Newton's gentian-violet-iodine.

The prophase stages are rather abnormal. The pollen mother cells show a great lethargy in meiotic divisions. In the same loculus pollen mother cells representing all stages from prophase to the second division and sometimes even tetrads were observed. At the first somewhat definable stage the pollen mother cells showed a nucleolus and chromosomes at a stage corresponding to mid-diplotene. The individual chromosomes could not be made out at this stage, so that the conjugation of the chromosomes had to be concluded from the metaphase observations only. The chromosomes, however, are seen to remain at this stage and never to enter a proper diakinesis at all. YAMASAKI was able, however, to distinguish a stage corresponding to the diakinesis with a bivalent and univalents.

The entry into the first metaphase is thus sudden. The univalents are found scattered all over the pollen mother cell, so that it is difficult to distinguish this stage from the first anaphase.

A bipolar spindle is regularly formed, though very often it is bent and occupies one side of the pollen mother cell. In such cases it appears to be much longer than the normal spindle. A centrally placed normal spindle is also fairly common. The shape of the spindle is to a great extent influenced by the position of the majority of the univalents in the cell. BLEIER (1933) has objected to the assertion made by DAVIS and KULKARNI that half-spindles occurred in the haploid of *Oenothera* investigated by them. By further researches on more material of the same species, BLEIER (l. c.) has shown that the previous authors were mistaken and had probably examined a cell that was cut through by the microtome knife. In the numerous cells examined in the present study not a single case with a half spindle was found. Half-spindles are never formed, even the most abortive of them were found to be bipolar.

An equatorial plate as such is not formed, though sometimes the univalents are found gathered there. The bivalents, however, are as a rule at the equatorial region. The equatorial region is thus found always to lie in the middle of the spindle and is not influenced by the position of the majority of univalents.

The univalents appear elongated. In the pollen mother cell three particular chromosomes could be distinguished, and they were found to be more or less constant:

1) One univalent with a large satellite-like body attached by a thick connection to the main chromosome (Fig. 1).

2) One univalent with a satellite somewhat widely separated and with a thin connection (Fig. 2).

3) One univalent with an achromatic portion in subterminal position. This chromosome has a normal median constriction. The achromatic part sometimes appears quite central, sometimes a little asymmetrical and in side views as though a portion of the chromosome was lost (Fig. 3).

In some of the pollen mother cells two univalents with satellites of the second type could be found. The satellite is sometimes found subterminally attached. Occasionally one of the univalents is found to be pointed without any other body attached (Fig. 4).

Very commonly the univalents are found sticking end to end. The number of univalents in such chains varies. This is probably due to

the abnormal prophasic stages and is of no importance. Such abnormalities were noticed by KIHARA in the haploid *monococcum* also.

Bivalent and trivalent associations are met with. The variation is given in Table 1. (Figs. 5—14).

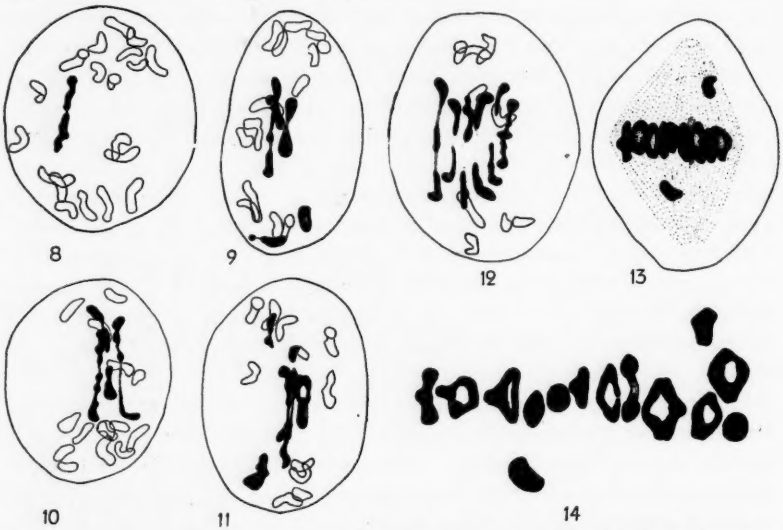

Fig. 8. $19_I\ 1_{II}$. (× 1500). — Fig. 9. $17_I\ 2_{II}$. (× 1500). One univalent with satellite and one with achromatic portion will become included in different nuclei. — Fig. 10. $15_I\ 3_{II}$. (× 1500). — Fig. 11. $11_I\ 5_{II}$. Two ring-bivalents probably interlocked (× 1500).

Fig. 12. $9_I\ 6_{II}$. The bivalents drawn a little separated from one another. (× 1500). — Fig. 13. $3_I\ 9_{II}$. Metaphase plate *in situ*. (× 1500). — Fig. 14. The bivalents drawn separated. The bivalent at the extreme left was shown to be a ring after the plate was flattened. (× 2100).

TABLE 1.

Kind of association	No. of P. M. C:s	Percentage	No. of cells with ring-bivalents
$21_I\ 0_{II}\ 0_{III}$	421	59,3	—
$19_I\ 1_{II}\ 0_{III}$	140	19,7	10
$17_I\ 2_{II}\ 0_{III}$	99	13,7	5
$15_I\ 3_{II}\ 0_{III}$	38	5,4	1
$13_I\ 4_{II}\ 0_{III}$	2	0,3	1
$9_I\ 6_{II}\ 0_{III}$	2	0,3	—
$3_I\ 9_{II}\ 0_{III}$	1	0,1	1
$18_I\ 0_{II}\ 1_{III}$	4	0,6	—
$16_I\ 1_{II}\ 1_{III}$	3	0,4	—
Total	710	100,0	—

YAMASAKI found up to 4 bivalents per pollen mother cell, but YAMAMOTO could find only up to 3. However, since 6 different types of bivalents occurred, the present author anticipated the possibility of finding all 6 bivalents in the same pollen mother cell. This happened in the present investigation (Fig. 12). As observed by YAMAMOTO, the chiasma was non-terminalised in only one of the bivalents. One bivalent with a large fragment-like portion attached by thin strands on either side to two large chromosome portions is not represented in his types (Figs. 5 and 12). This bivalent might often be mistaken for a trivalent. But one of the gemini has a large submedian constriction, and during anaphase this becomes stretched, so that the central portion becomes thin and the tip portion appears as a separate one. In other cases the anaphase stretching is so great that only a small faintly stained chiasma is found attached by very thin plasma strands to two large chromosome bodies.

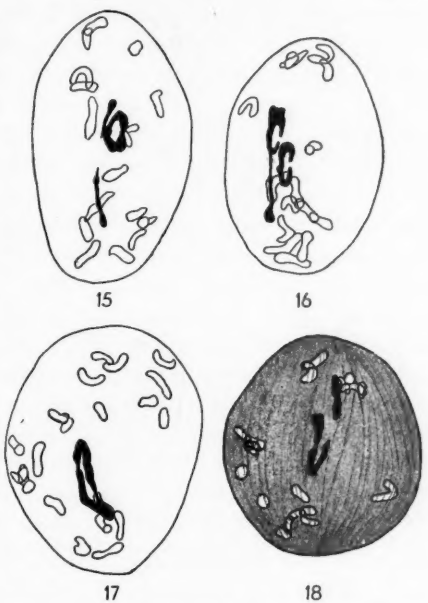

Fig. 15. One ring- and one ordinary bivalent and 17_I. (× 1500). — Fig. 16. Two ring-bivalents at anaphase, also one ordinary bivalent and 15_I. (× 1500). — Fig. 17. 18_I 1_{III}. (× 1500). — Fig. 18. 18_I 1_{III} at anaphase. (× 1500).

The greatest number of bivalents formed was 9 (Figs. 13 and 14). Only one pollen mother cell, showing such unexpected high pairing affinity in the chromosomes, was found, though many hundreds were examined in the course of the investigation. The cell shows a metaphase plate as in a normal diploid plant with a few univalents. The plate is regular and compact. Of the nine bivalents, eight were found to be ring-shaped and one rod-shaped. Three definite univalents and two spherical bodies are also represented in Fig. 14. What these two spherical bodies are could not be understood. They become apparent only at the topmost and the lowermost focussing. An attempt to flatten

the plate in order to get a clearer view of the bivalents resulted only in demonstrating that one of the apparent rod-like bivalents with a non-terminalised chiasma was a ring-bivalent. For fear of damaging the preparation, further flattening was not tried. It is probable that they are only the polar views of the ends of the ring-bivalents.

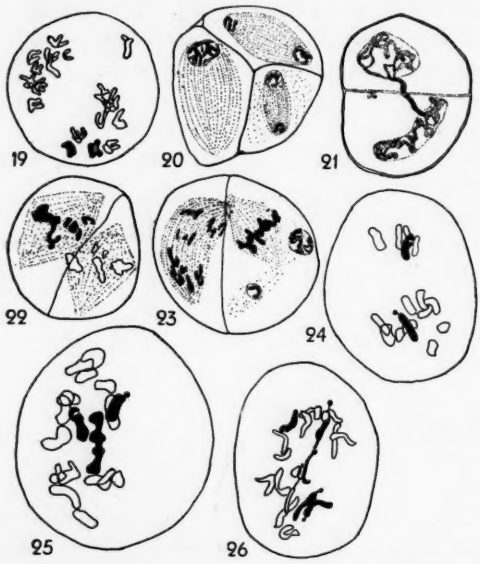

Fig. 19. Univalents showing lengthwise splitting at the end of the first division. (× ca. 1170). — Fig. 20. Three cells formed at the end of the first division. Second division walls not yet formed. The daughter nuclei beginning to disorganise. (× ca. 1125). — Fig. 21. One chromosome cut into two by the cell wall. (× ca. 1125). — Fig. 22. Second metaphase plates. (× ca. 1125). — Fig. 23. Second ana- and metaphase, showing two micronuclei at resting stage, about which spindles have formed. (× ca. 1125). — Fig. 24. The achromatic and satellite chromosomes at opposite poles. A chain of two chromosomes showing end to end attachment. (× 1500). — Fig. 25. The same two univalents at the same pole. (× 1500). — Fig. 26. Cell showing two satellite chromosomes and one with achromatic portion. (× ca. 1125).

The trivalents are of the same type as that described by the previous authors. Only one per cell is found (Figs. 7, 17 and 18).

Ring-bivalents like those reported by YAMASAKI were found. He, however, was able to observe only one per cell. In the present case 2 ring-bivalents in the same pollen mother cell were found and, as already mentioned, in the extreme case 8 (Figs. 6, 15 and 16). Two possible instances of interlocking were met with. These were simple interlocking of two separate rings (Fig. 11).

At anaphase, as reported by the previous workers, the univalents are distributed wholly at random. They are usually included into the pole to which they lie nearest. The univalents found at the equatorial plate are rarely undergoing an equational division. The bivalents on the other hand undergo regular separation. Sometimes a complete bivalent may be included

in the same interphase nucleus. The end of the anaphase is often characterised by the univalents splitting lengthwise (at this stage the first metaphase spindle alone is found). (Fig. 19). The same random distribution of the chromosomes to the poles as that reported by YAMAMOTO was also found in the present case.

The interphase is very often normal. Two daughter nuclei are formed. Restitution nuclei are commonly found. In one case a chromosome delayed in separation was found cut into two by the formation of the cell wall (Fig. 21). Triads are also sometimes found at the end of the first division (Fig. 20).

All the interphase nuclei undergo a second division. The spindles are formed quite irrespective of the number of chromosomes, but the size of the spindle depends on the number. Without exception the spindles formed are bipolar. Sometimes weak attempts at forming spindles even about micronuclei, whose chromosomes have not even made an attempt to go into the metaphase condition, are found (Fig. 23).

Such of the cells as are free from micronuclei form a normal metaphase plate, though it lacks the compactness and orderliness found in a diploid plant. The anaphase and telophase also run normal. In other cases the formation of the plates are disturbed by the smaller spindles. In some cases 3—4 spindles and separate plates are found. Frequently the pollen mother cells are found degenerating without even completing their second division (Figs. 20, 22 and 23).

Tetrads are formed with remarkable regularity. Triads and more than four nuclei are also often met with.

TABLE 2.

Species	n	Percentages of bivalent formations								Author
		0	1	2	3	4	6	9	1_{III}	
Tr. monococcum	7	98,0	2,0	—	—	—	—	—	—	KIHARA and KATAYAMA
Tr. durum	14	84,8	13,9	11,2	0,08	—	—	—	—	KIHARA
Tr. vulgare	21	47,7	37,1	2,4	2,4	0,4	—	—	(Taken as one bivalent)	YAMASAKI
»	21	86,1	10,5	1,8	0,1	—	—	—	0,1	YAMAMOTO
»	21	59,3	19,7	13,9	5,3	0,3	0,3	0,1	1,0	The present investigation

Table 2 gives the percentages of bivalent and trivalent formation in the haploids of the diploid, tetraploid and hexaploid wheats. It will be seen that the tendency to pair increases with the multiplication of the genomes. In the *vulgare* groups the percentage of univalents and bivalents varies. This is probably due to the time of observation. More univalents are counted at later metaphases than at the earlier ones. KIHARA (in KIHARA and NISHIYAMA, 1930) found that external conditions to a certain extent also influence the pairing in haploids and hybrids. It will also be seen that 1—2 bivalents per cell occur with a greater frequency than the higher numbers. This possibly is due to the different degrees of homologies between the chromosomes of the haploid set.

IVANOV (1938, p. 369), in reviewing the wheat haploids, remarks: »It is also of interest that there were never seven bivalents (the basic chromosome number of *Triticum*) but always less (not more than 4) . . .». Thus he seems to expect the highest number of bivalent formation to be limited to the basic number. However, the configuration with the highest number of paired chromosomes possible in the hexaploid wheat is $1_I + 10_{II}$ or $9_{II} + 1_{III}$, tetravalents never having been met with.

But KIHARA and NISHIYAMA (1930), from their crossing experiments, have come to the conclusion that the hexaploid wheats have the genomes A, B and D. Their triploid hybrid has shown that auto- as well as allosyndesis may occur between the three genomes (A) + + (A + B). The behaviour of the tetraploid hybrid (A) + (A + B + D) led them to conclude again a stronger affinity between 1—2 chromosomes of the A genomes with that of the B and 3 from B with the D genomes. With the possibility of the trivalent-forming chromosome being separate from the above 5 it would justify the building of 6 bivalents. The rest of the bivalents have risen probably owing to allosyndesis between A and B genomes.

It is rather remarkable that in the various bivalent formations not a single case was found in which the chromosome paired with the achromatic portion. The satellite-chromosomes, on the other hand, were found in some cases to form bivalents but it could not be said with certainty whether both chromosomes had satellites or not. When univalent these chromosomes undergo the same random distribution as the others (Figs. 24, 25 and 26). In the case of the pollen mother cells with 6 and 9 bivalents it could not be decided whether they formed bivalents or not, owing to the fact that some of the univalents were not lying flat in one plane. No mention of these chromosomes has been made either

in connection with the hybrids or haploids by the other authors. SIMONET and FLECKINGER (1937), however, describe two chromosomes with a large subterminal constriction in the diploid and one such chromosome in the haploid. The position and shape of this constriction, as judged from their drawings, is similar to the achromatic portion found in one of the univalents in the present haploid plant. It is highly probable that this, too, is a subterminal constriction and the genome of *Tr. vulgare* has also only one such chromosome.

SUMMARY.

The meiotic behaviour in the pollen mother cells of a haploid plant of *Triticum vulgare* is described. The chromosomes were found to form bivalents and trivalents. The highest number of bivalents found per cell was 6 and in one solitary case 9. Only one trivalent per cell was found. The investigations of KIHARA and others have justified the occurrence of 6 bivalents. The extra three bivalents in the 9 bivalent cell is referred to allosyndesis between the A and B genomes of the A, B and D genomes of *Tr. vulgare*. Three particular univalents with distinguishing characters are described. It is suggested that these may help in the further analysis of the genomes of this plant.

The above investigations were carried out at the Cyto-genetic Laboratory of the Sveriges Utsädesförening, Svalöf. The somatic chromosomes were studied by Miss M. PALM and the fixations of the meiotic stages were done by Miss H. NORDENSKIÖLD, to both of whom my best thanks are due. My grateful thanks are due to Professor A. MÜNTZING for kindly permitting me to work on this material and also for his kind interest and criticisms throughout the work. My sincere thanks are due to the Director for his kindness in permitting me to work at this Institute.

Svalöf, Sweden, September 20th, 1938.

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CYTOLOGICAL PHENOMENA CONNECTED WITH THE ROOT SWELLING CAUSED BY GROWTH SUBSTANCES

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AS is well known the influence of growth substances on roots is quite different from that on shoots. Almost all concentrations, with exception of the most diluted ones, inhibit root elongation. In addition to this effect high concentrations bring about very characteristic swellings of the roots. As these formations show a striking resemblance to the colchicine tumours, which have been shown to exhibit very interesting cytological features (LEVAN, 1938), I considered it worth while making a cytological study also of the root swellings caused by growth hormones. I decided to use the same material as in my colchicine work, *viz.* root tips of *Allium*, mainly on account of their excellent cytological conditions.

Experimental series were planned and arranged in co-operation with Dr. G. BORGSTRÖM, Lund, who will publish the physiological results of this investigation. The species mostly used were *Allium Cepa* and *fistulosum*. The following substances were tried: Indolyl-3-acetic acid; Indolyl-3-butyric acid; Naphthalene-1-acetic acid; Phenyl-acetic acid; Phenyl-propionic acid. — For the detailed arrangement of the experiments the reader is referred to BORGSTRÖM (1939). Dilutions of from 1000 to 0,001 ppm (parts per million) were employed. The best cytological results were obtained in the concentrations 10 and 1 ppm, which gave a striking effect without killing the roots.

NAVASHIN's fluid was used for the fixing. Longitudinal and transverse sections, 20 μ in thickness, were cut and stained in gentian violet. My thanks are due to Miss ANNA NORDSTRAND, who prepared the slides for this investigation.

I. HISTOLOGICAL RESPONSE OF THE ROOTS.

All the growth substances employed had in common an inhibiting effect on the root elongation. This effect is of a different strength in different substances. Most extreme in their effect are naphthalene-

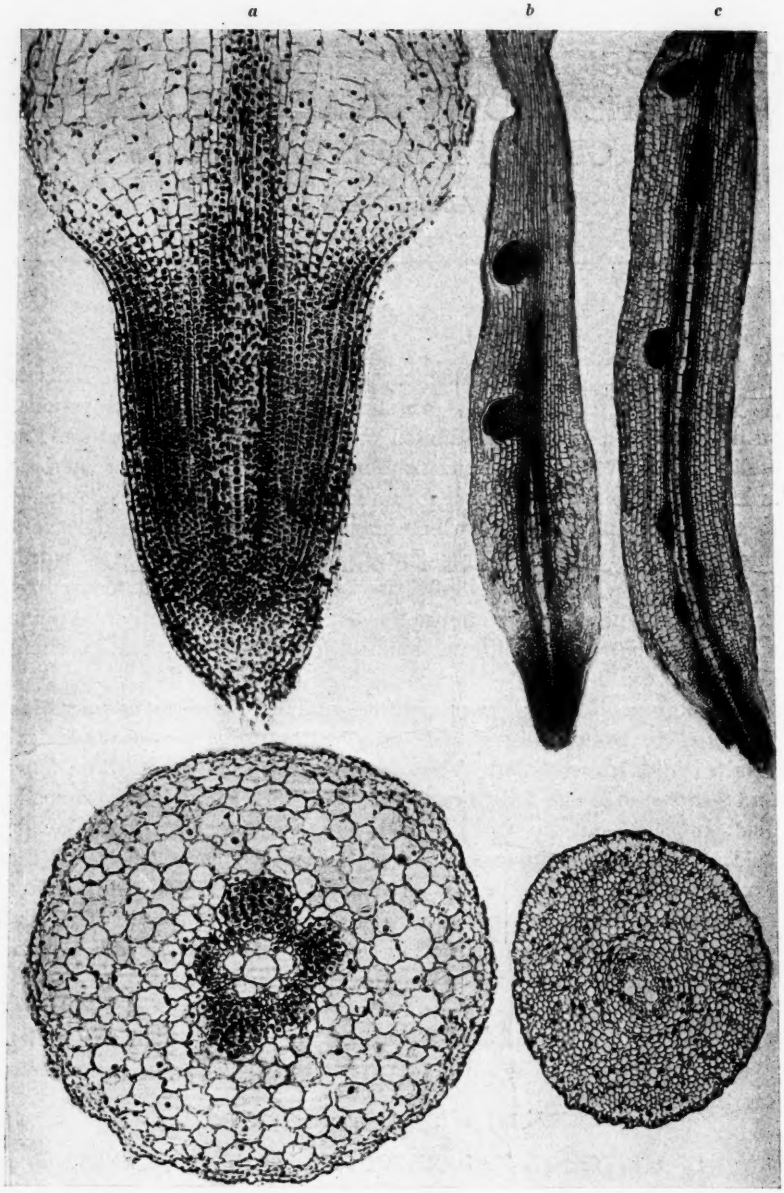


Fig. 1. Microphotographs of root tips, *a*—*c* longitudinal sections, *d*—*e* transverse sections. *a*: 1 ppm Indolyl-butyric acid treated for 4 days, *b*: 1 ppm Naphthalene-acetic acid for 6 days, *c*: ditto for 3 days, *d*: ditto for 3 days, *e*: untreated root, section from the same level of the root as *d*. — *a*, *d*, *e*, × 50, *b*, *c*, × 16. Microphoto OTTO MATTSSON.

acetic acid and indolyl-butyric acid, both of which effectively inhibit the longitudinal growth of the roots in the concentrations 1—10 ppm, while the swelling of the roots is very conspicuous. Other substances, for instance phenyl-acetic acid and phenyl-propionic acid, cause only retardation of the root elongation.

In a longitudinal section of a thickened root (Fig. 1 *a*) two clearly separated regions may be distinguished: 1. the narrow root tip with lively divisions taking place in the apical meristem, 2. the upper thickened part of the root, where the cortex consists of giant cells. Fig. 1 *d* and *e* furnish a comparison between two transverse sections from about the same level of one treated and one untreated root. It is evident that the enlargement of the cortical cells is caused by the treatment, and the root tumours are mainly due to the swelling of the cortical cells.

The increase in cell volume varies somewhat following treatment with different substances. This is dependent on the direction of the maximal enlargement of the cells. If this direction is transversal, the root tumours will be more pronounced and the transition between the tip and the thickened part of the root will be more abrupt.

Fig. 1 *a* elucidates this condition. It is possible here to follow the cell rows from the tip meristem upwards. The transition from small meristematic cells to the enlarged cells takes place within a range of 5—10 cells. Thus it is clear that the increase in volume is effected by a real growth of the cells and not by cell fusions, with or without mitoses.

On the other hand it will be seen from the longitudinal section that a larger number of cell rows is present than normally. This is due to the fact that the meristematic cortical cells under the influence of the growth substances start numerous mitoses. These mitoses are orientated in the transverse axis of the root, tangentially or radially. Each parenchyma cell is in that manner divided first into two and then into four cells. This phenomenon may be described as a change in polarity of the cells, since in untreated material nearly all divisions are orientated longitudinally. These extra divisions, however, are not a necessary condition for the subsequent swelling of the cells. Frequently the divisions are missing, and the cortical parenchyma then consists of rows of simple cells, which after a certain time interval begin to swell in the ordinary manner.

After a treatment of 4—6 days the mitoses of the apical meristem decrease in number and soon the cells cease dividing altogether. Often

the tip atrophies but even after a treatment of 8 days it may start growing again, if transferred into water.

At the same time as the tip meristem ceases its activity lively mitoses take place farther off from the apex in the exterior layers of the central cylinder. These divisions may be equally dispersed along the entire surface of the central cylinder, which will then be covered by several layers of small meristematic cells, forming a striking contrast to the surrounding large-celled cortex. Usually, however, limited portions of meristematic tissue are formed at certain distances along the central cylinder, giving rise to lateral roots (Fig. 1 *b—d*).

Thus the growth substances in the concentrations used have the following effects on the roots:

1. An increase in the number of the mitoses in the apical meristem, connected with a change of the cell polarity.

2. Starting of numerous mitoses in the pericycle resulting in the formation of lateral roots.

3. An increase in volume of the cortical cells at a certain stage of their development.

II. CYTOLOGICAL RESPONSE OF THE ROOTS.

As already found by BEAL (1938) in *Lilium*, the mitoses of the meristematic cells run a normal course after treatment with growth substances. »The observations indicate that the mitotic figures are as distinct as in the cells of roots or stem tips taken from plants grown under ordinary garden conditions, and lead to the conclusion that the divisions are perfectly regular in all respects» (l. c. p. 906).

This is true concerning most meristematic cells in *Allium* too. In *Allium*, however, the enlarged cortex tissue exhibited cytological conditions of the very greatest interest. First of all the mere occurrence of divisions in this tissue is rather extraordinary. In fact, mitoses were found at a distance of 3 mm from the apex, a condition never present in this tissue of untreated material. The chromosome number of these mitoses varies according to the location in the root. Close to the apical meristem the number of chromosomes is normal, $2x = 16$, while farther off from the apex the majority of cells show 32 chromosomes. Still more distant from the apex the number 64 dominates.

An idea of these conditions may be had from Table 1, which gives an instance of the distribution of chromosome numbers within the cortex. The table is compiled from all the transverse sections of 3

roots. From the table it will be seen that there exists a definite correlation between the increase in chromosome number and the location in the cortex tissue. The most simple explanation of these conditions would seem to be that the increase in chromosome number precedes and causes the increase in cell volume, in the same manner as has been shown in colchicine treated material, where similar large-celled tumours are formed by the cortex.

TABLE 1. *Chromosome numbers of the giant cells of the cortical parenchyma.*

Distance from the apex	Root 1			Root 2		Root 3	
	16	32	64	16	32	16	32
0— 500 μ	1	0	0	—	—	1	0
501—1000	4	2	0	—	—	2	3
1001—1500	2	9	0	2	1	1	12
1501—2000	0	14	4	1	3	—	—
2001—2500	0	6	11	2	21	—	—
2501—3000	—	—	—	0	11	—	—

A closer examination reveals, however, that the situation is entirely different. As mentioned, there occurs in the meristem, which of course is also under the influence of the growth hormones, numerous mitoses, which are completely normal both with respect to the chromosome number and the course of the division. A similar effect of the growth substances to that of colchicine is consequently excluded. Further, the early divisions of the giant cortical cells are almost without exception regularly diploid, not until the giant cells have reached a certain age do the doublings begin to show up. It is evident that the growth in volume of the cells is a reaction primarily caused by the growth substances, the increase in chromosome number is a secondary phenomenon.

The mechanism of the origin of these chromosome doublings is quite different from the mechanism of the colchicine-induced doublings. Colchicine influences the most active stages of the mitoses, it interferes with the anaphase movement of the chromosomes. On the other hand, the doublings we are now discussing take place during the resting stage, and are consequently independent of the activity of the mitotic process. As this mechanism of chromosome doubling has not been previously reported from the plant kingdom, it will be described in some detail.

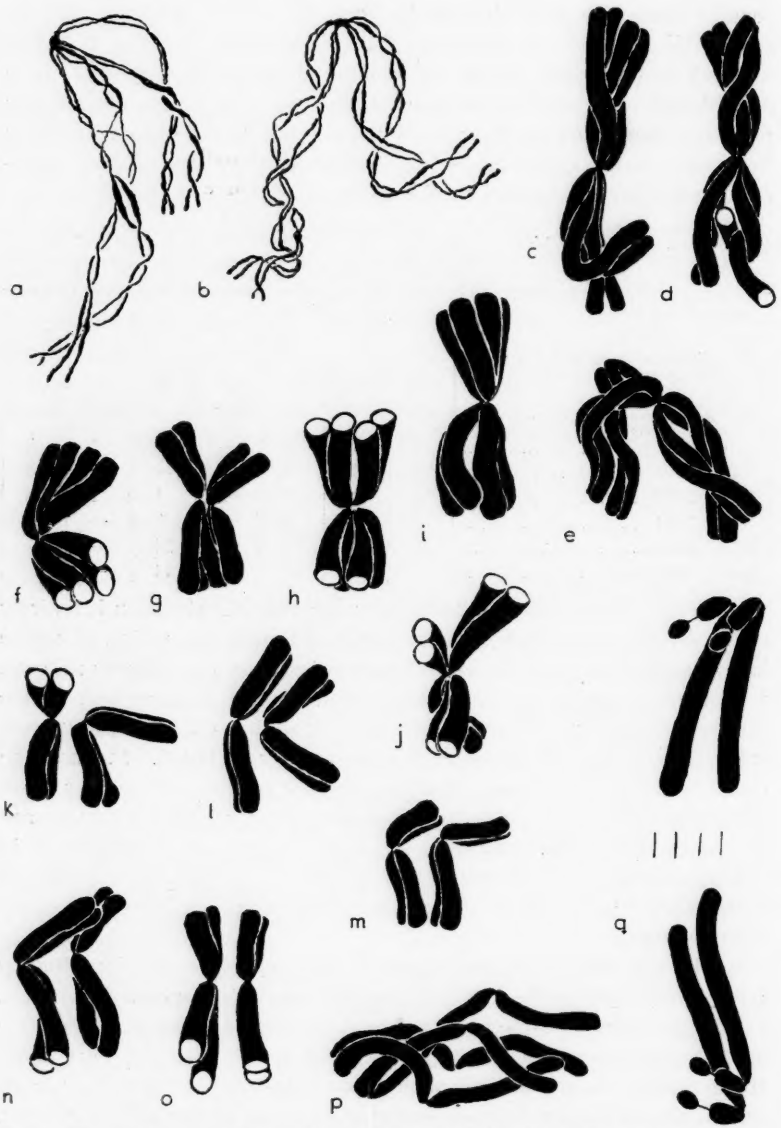
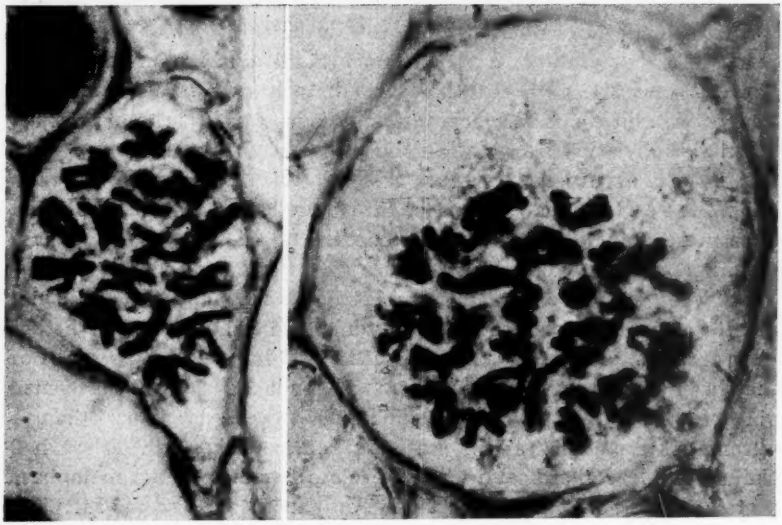


Fig. 2. The mechanism of chromosome doubling in the giant cells. *a*, *b*: prophase, *c*—*j*: metaphase, the centromeres are single, *k*—*o*: ditto, the centromeres are divided, *p*: early anaphase, *q*: late anaphase. — × 3900.

As early as the prophase chromosomes can be distinguished they show a quadripartite structure instead of the usual bipartite structure. The four threads form 2 minor relational spirals, which are coiled together into one relational spiral of a higher order. On a few occasions I have observed exchanges of threads between the 2 minor spirals (for instance, in Fig. 2 *a*), but owing to the difficulty in observation of these stages I do not dare to decide whether real chiasmata occur or whether it is a question of an interlocking or simply sticking together



a *b*

Fig. 3. Microphotographs of metaphase in doubled cells. *a*: cell with 32 chromosomes within the central cylinder. *b*: giant cell with 64 chromosomes in the cortex. — × 1500. Microphoto OTTO MATTSSON.

of threads. The centromeres are undivided during the prophases, and each centromere joins 4 chromatids in a similar manner to that of the diplochromosomes described by WHITE (1935). The singleness of the centromere is the main difference between these prophases and the meiotic prophases, and the whole character of the division is determined by this feature. Instead of the large central loop of the meiotic bivalents, due to the specific repulsion of the centromeres, there is formed here one large loop on each side of the undivided centromere (Fig. 2 *a*—*b*). During the subsequent contraction of the chromosomes, the original spirals uncoil, first the major spiral, then the minor one.

During this uncoiling of the spirals the four chromatids are lying close together (Fig. 2 *c*—*e*). The still undivided centromere prevents an eventual repulsion between pairs from becoming visible, at any rate in the central parts of the chromosomes.

The nuclear membrane disappears and the 16 diplochromosomes are arranged in the equatorial plate (Fig. 3 *a*). The spindle functions normally even in the oldest cells. The quantity of plasm in these cells is often very small in relation to their size. The plasm often forms just a thin layer along one wall. In this layer the equatorial plate is then situated, often rather excentrically in the cell. The chromosomes reach their greatest contraction and the 4 chromatids are lying parallelly (Figs. 2 *f*—*j*). At the same time it may be seen that the centromeres have been divided, although it is impossible to decide just when this division takes place. The number of chromosomes is in this way doubled and the chromosomes remain arranged in pairs (Fig. 2 *k*—*o*). Immediately afterwards the centromeres are divided once more and then the anaphase movement sets in. The anaphase (Fig. 2 *p*—*q*) and telophase take place quite regularly.

Owing to the relative scarcity of the octoploid plates I was not able to study their prophases. Consequently I cannot decide whether they originate directly from diploid cells by 3 longitudinal divisions of the chromosomes during the resting stage, or if they have been formed via tetraploid mitoses. In metaphase, however, not more than 4 chromatids are joined at the same centromere (Fig. 3 *b*), although 2 similar diplochromosomes usually lie close together in the metaphase plates, a condition which is detectable only in the case of the satellited chromosomes.

III. DISCUSSION.

The most remarkable feature, from a theoretical point of view, among the modifications, described above, of the root development caused by the growth substances is undoubtedly the mechanism of chromosome doublings in old cells. This mechanism of chromosome doubling is unparallelled in the plant kingdom. The conclusion is drawn that the cell enlargement in itself is the stimulus to the doubling. It must be mentioned here that also cells within the central cylinder have shown, though seldom, the same kind of chromosome doubling. This has, however, always been certain of the normally very large cells of the vascular tissue.

Then the very important question arises as to whether, in un-

treated material, tissues growing mainly by cell enlargement without cell divisions may also contain doubled or multiplicated chromosome complements. It is usually considered as an axiom that all the somatic cells of an individual have the same genic quantity. The conditions of the large cortical cells in *Allium* show, however, that this is not always the case, even if it cannot be decided in most cases, since such cells very seldom divide.

In this connection HERTWIG's (1935) studies may be recalled. He points out that the growth of cells often occurs rhythmically and discontinuously by doublings of their volume. Without any possibility of actually counting the chromosomes in such material he sets up the theory that after the doubling of the cell volume the chromosomes of the cells are »mitosebereit». And if the mitosis from any reason is prevented from occurring, the chromosomes undergo an »innere Teilung», a process which may be repeated several times, giving rise to 2-, 4-, 8-, and so on valent chromosomes.

Such cells with a very great increase in volume during the development are occasionally found in animal material. Well known are the salivary gland cells of the Diptera. It is generally agreed that their giant chromosomes originate by repeated divisions of the chromonema during the resting stage, although there is some diversity of opinion concerning the final number of chromonemata present.

An especially interesting case of chromosome duplication during the resting stage has recently been described by BERGER (1938 a, b and elsewhere). In different species of *Culex* the cells of the iliac epithelium grow during the larval period from a diameter of 3—4 μ to 10,4—17,5 μ. After pupation these large cells begin to divide and it appears then that the chromosome number has increased during the period of cell enlargement from the normal number $2n = 6$ to 48, 96 or even 192 chromosomes. In this connection the recently published (BERGER, 1938 b) description of the prophases of this first post-larval division are of special interest, since it constitutes the only existing parallel to the prophases of the giant cells of *Allium*. In the earliest observable prophase of *Culex* the chromosomes appear in 6 bundles. This condition is explained as »evidence that the initial synapsis unites sister threads not homologous ones». As the prophase advances the bundles fall apart into units, »the unit being a synapsed pair of sisters in the first division».

It is clearly seen from the accompanying microphotographs (for instance, 1938 a, Figs. 17—19) that these »synapsed pairs» constitute

2 half-chromosomes joined at the still undivided centromere and held together by what remains of the relational coiling. Thus they are comparable to the Fig. 2 *k*—*o* of the present paper. They are arranged in pairs in the plate, just as in *Allium*.

Concerning the possibility of this mechanism for inducing polyploidy it may be said that its success is entirely dependent on whether such doublings may be effected in cells which have any chance of growing out into new shoots. Tetraploid mitoses without the characteristic diplochromosomes have now and then been met with in the cortex. They are evidently later generations of mitoses in previously doubled cells, and they show that normal tetraploid mitoses may result from this process of doubling.

This study of *Allium* is limited to root tissues but there is no reason to suppose that the same cytological laws are not valid also in stem tips. GREENLEAF (1937) has induced polyploidy in *Nicotiana* by applying a paste of hetero-auxin on the cut surface of decapitated shoots. The possibility cannot be entirely excluded that cells, which under the influence of hetero-auxin have become enlarged and subsequently chromosome-doubled, may participate in the formation of callus shoots.

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